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REVISED INTERIM UTILITY GUIDELINES TRAINING MATERIALS

Contents

SYNOPSIS OF APPLICATION OF THE REVISED INTERIM UTILITY GUIDELINES

It is assumed at this point in the analysis that the specification has been reviewed and an appropriate search of the claimed subject matter has been conducted. It is also assumed that some "utility" is disclosed in the specification or is recognized to be well-established in the art. The examiner should determine whether any asserted utility is specific and substantial, and if so, determine whether such asserted utility is credible. In determining credibility the examiner should consider whether or not there currently are similar or equivalent materials and/or procedures available for achieving that utility. If there are, the utility is credible and no rejection under 35 U.S.C. § 101 should be made.

Guidance for Various Examination Situations

I) a) For method claims that recite more than one utility, if at least one utility is credible, specific, and substantial, a rejection under 35 U.S.C. § 101 should not be made. If any utility in such a claim is not a specific and substantial credible utility, i.e., the claim encompasses at least one utility that does not meet the requirements of 35 U.S.C. §101, the rejection of the claim should be addressed under 35 U.S.C. § 112, first paragraph, scope of enablement.

b) For product claims that do not recite any utilities, disclosure or assertion of one specific, substantial and credible utility meets the criteria of 35 U.S.C. § 101.

II) If no credible, specific, and substantial utility is asserted in the specification and none is well established, a rejection under 35 U.S.C. § 101 would be proper.

III) Cure or prevention - Utilities that constitute curing or preventing a condition are sometimes not credible to one of skill in the art and thus may raise a question under 35 U.S.C. § 101. However, any rejection based on lack of credible utility must be supported by documentary evidence or sound technical reasoning.

IV) Treatment - Since most diseases or conditions can be treated, rejections under 35 U.S.C. § 101 for treatment claims should rarely be made.

V) Vaccines - Since vaccines are regularly prepared to combat various viruses and organisms, vaccines would have a credible utility to one of skill in the art. Thus, vaccines, including those for small pox, should not raise a question under 35 U.S.C. § 101.

VI) Materials to be used for research, or methods of using those materials for research, raise issues of whether the utilities require or constitute carrying out further research to identify or reasonably confirm a "real world" context of use. See, e.g., Brenner v. Manson, 383 U.S. 519, 148 USPQ 689 (Sup. Ct. 1966) wherein a research utility was not considered a "substantial utility."

Definitions

"Credible utility" – Where an applicant has specifically asserted that an invention has a particular utility, that assertion cannot simply be dismissed by Office personnel as being "wrong". Rather, Office personnel must determine if the assertion of utility is credible (i.e., whether the assertion of utility is believable to a person of ordinary skill in the art based on the totality of evidence and reasoning provided). An assertion is credible unless (A) the logic underlying the assertion is seriously flawed, or (B) the facts upon which the assertion is based are inconsistent with the logic underlying the assertion. Credibility as used in this context refers to the reliability of the statement based on the logic and facts that are offered by the applicant to support the assertion of utility. A *credible* utility is assessed from the standpoint of whether a person of ordinary skill in the art would accept that the recited or disclosed invention is currently available for such use. For example, no perpetual motion machines would be considered to be currently available. However, nucleic acids could be used as probes, chromosome markers, or forensic or diagnostic markers. Therefore, the credibility of such an assertion would not be questioned, although such a use might fail the *specific* and *substantial* tests (see below).

"Specific utility" – A utility that is *specific* to the subject matter claimed. This contrasts with a *general* utility that would be applicable to the broad class of the invention. For example, a claim to a polynucleotide whose use is disclosed simply as a "gene probe" or "chromosome marker" would not be considered to be *specific* in the absence of a disclosure of a specific DNA target. Similarly, a general statement of diagnostic utility, such as

diagnosing an unspecified disease, would ordinarily be insufficient absent a disclosure of what condition can be diagnosed.

"Substantial utility" - a utility that defines a "real world" use. Utilities that require or constitute carrying out further research to identify or reasonably confirm a "real world" context of use are not substantial utilities. For example, both a therapeutic method of treating a known or newly discovered disease and an assay method for identifying compounds that themselves have a "substantial utility" define a "real world" context of use. An assay that measures the presence of a material which has a stated correlation to a predisposition to the onset of a particular disease condition would also define a "real world" context of use in identifying potential candidates for preventive measures or further monitoring. On the other hand, the following are examples of situations that require or constitute carrying out further research to identify or reasonably confirm a "real world" context of use and, therefore, do not define "substantial utilities":

A. Basic research such as studying the properties of the claimed product itself or the mechanisms in which the material is involved.

B. A method of treating an unspecified disease or condition. **(Note, this is in contrast to the general rule that treatments of specific diseases or conditions meet the criteria of 35 U.S.C. § 101.)**

C. A method of assaying for or identifying a material that itself has no "specific and/or substantial utility".

D. A method of making a material that itself has no specific, substantial and credible utility.

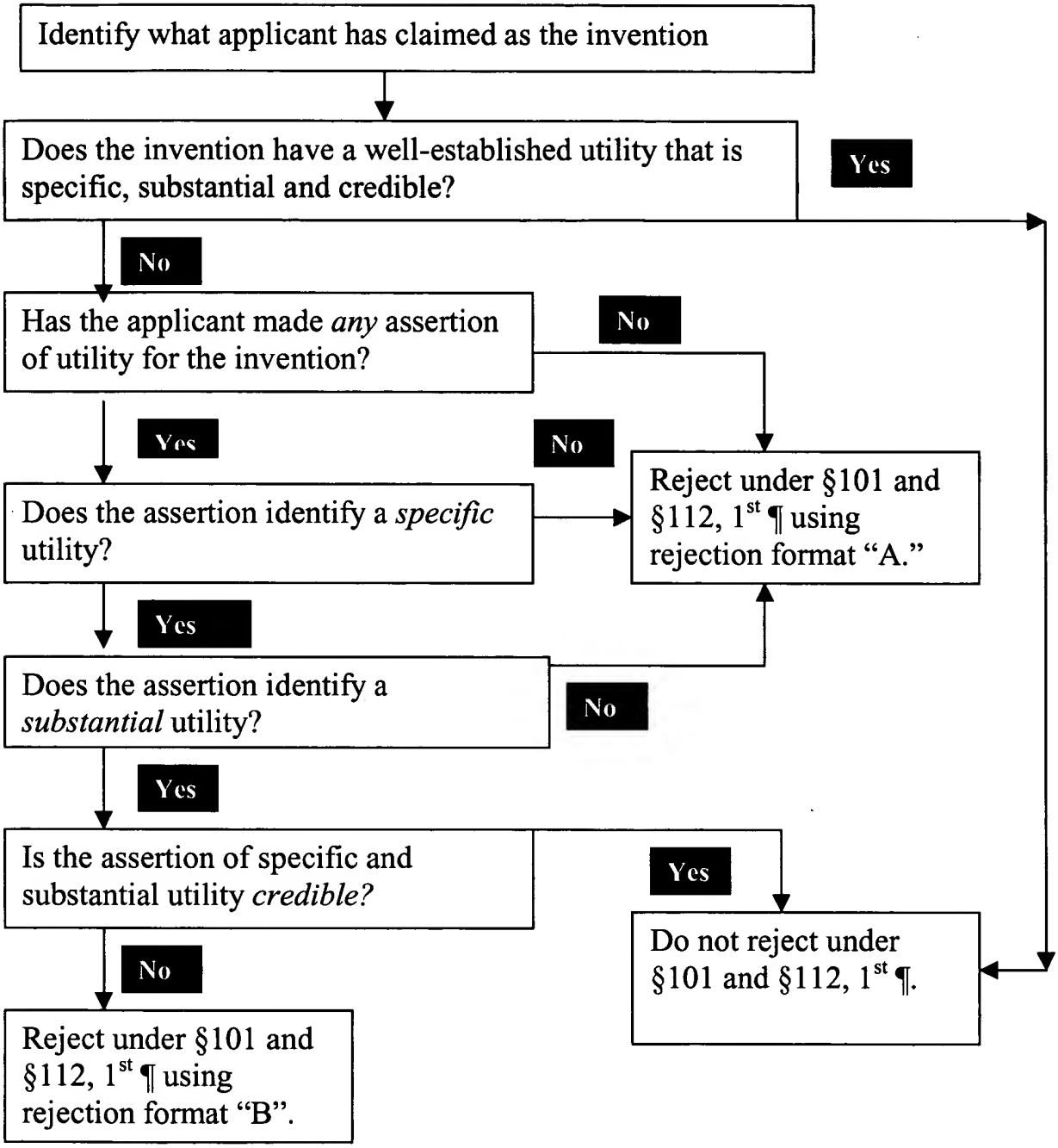
E. A claim to an intermediate product for use in making a final product that has no specific, substantial and credible utility.

Note that "throw away" utilities do not meet the tests for a *specific* or *substantial* utility. For example, using transgenic mice as snake food is a utility that is neither specific (all mice could function as snake food) nor substantial (using a mouse costing tens of thousands of dollars to produce as snake food is not a "real world" context of use). Similarly, use of any protein as an animal food supplement or a shampoo ingredient are "throw away" utilities that would not pass muster as specific or substantial utilities under 35 U.S.C. §101. This analysis should, of course, be tempered by consideration of the context and nature of the invention. For example, if a transgenic mouse was generated with the specific provision of an enhanced nutrient profile, and disclosed for use as an animal food, then the test for specific and substantial *asserted* utility would be considered to be met.

"Well established utility" - a specific, substantial, and credible utility which is well known, immediately apparent, or implied by the specification's disclosure of the properties of a material, alone or taken with the knowledge of one skilled in the art. "Well established utility" does not encompass any "throw away" utility that one can dream up for an invention or a nonspecific utility that would apply to virtually every member of a general class of materials, such as proteins or DNA. If this were the case, any product or apparatus, including perpetual motion machines, would have a "well established utility" as landfill, an amusement device, a toy, or a paper weight; any carbon containing molecule would have a "well established utility" as a fuel since it can be burned; and any protein would

have well established utility as a protein supplement for animal food. This is not the intention of the statute.

Utility Review Flowchart



Rejection format "A": **Applicant has not disclosed any specific and substantial utility for the claimed invention, credibility will not be assessed.**

Rejection format "B": **Applicant has disclosed at least one specific and substantial utility for the claimed invention, but the assertion is not credible.**

Form Paragraph

7.05.01 - UTILITY REJECTIONS UNDER 35 U.S.C. § 101 AND 35 U.S.C. 112, FIRST PARAGRAPH

Claim [1] rejected under 35 U.S.C. § 101 because the claimed invention is not supported by either a [2] asserted utility or a well-established utility.

[3]

Claim [4] also rejected under 35 U.S.C. § 112, first paragraph. Specifically, since the claimed invention is not supported by either a [5] asserted utility or a well established utility for the reasons set forth above, one skilled in the art would not know how to use the claimed invention so that it would operate as intended without undue experimentation.

Format A: No specific and substantial utility

a) Insert the same claim numbers in brackets 1 and 4.

b) Insert "specific and substantial" in brackets 2 and 5.

c) In bracket 3, insert the explanation as to why the claimed invention is not supported by a specific and substantial asserted utility or a well-established utility. Note in the office action that credibility will not be assessed.

d) Format A is to be used either when there is no asserted utility or when there is an asserted utility that is not specific and substantial.

Format B: No credible utility

a) Insert the same claim numbers in brackets 1 and 4.

b) Insert "credible" in brackets 2 and 5.

c) In bracket 3, insert the explanation as to why the claimed invention is not supported by either a credible asserted utility or a well-established utility. Note that a utility that is inoperative is not credible.

Format C: For claims that have multiple utilities, some of which are not specific and/or substantial, and some of which are not credible, but none of which are specific, substantial and credible:

a) Insert the same claim numbers in brackets 1 and 4.

b) Insert "specific and substantial asserted utility, a credible" in brackets 2 and 5.

c) In bracket 3, insert the explanation as to why the claimed invention is not supported by a specific and substantial asserted

utility, a credible asserted utility or a well-established utility. Each utility should be addressed.

UTILITY GUIDELINES: TRAINING EXAMPLES

Example 1: Alternative Uses Claimed

Specification: The specification relates to the prevention and treatment of microbe X infection, a common infection, by administering compound A.

Claim:

1. A method for preventing or treating microbe X infection comprising administering to an animal in need thereof an effective amount of compound A.

2. A method for preventing microbe X infection comprising administering to an animal in need thereof an effective amount of compound A.

Analysis: The following analysis includes the questions that need to be asked according to the guidelines and the answers to those questions based on the above facts:

1) Based on the record, is there a "well established utility" for the claimed invention? Since each claim is directed to a specific method of use, the utility of each of these claims is limited to that use and the examiner should not look to a "well established utility" for the composition used in the claimed method. Consequently, the answer to the question is no.

2) Has the applicant made any assertion of utility for the specifically claimed invention? Yes. In fact, for claim 1 there are two asserted utilities, i.e., preventing microbe X infection and treating microbe X infection. Since there are two asserted utilities for claim 1, each must be analyzed. For claim 2, the utility is preventing microbe X infection.

3) Is the asserted utility specific? Since microbe X infection is a known infection, and the treatment claimed is directed to a *particular* combination of treatment and agent, the utilities of preventing or treating the infection define specific and particular uses, and are therefore specific utilities.

4) Is the asserted utility substantial? The characterization of the disease as a common infection establishes the presumption that the asserted utilities have a "real world" context. Therefore, the asserted utility is substantial.

5) Is the asserted specific and substantial utility credible? Since infections are conventionally treatable, the answer to this question would be yes regarding the treatment of microbe X infection. However, the claims also recite preventing microbe X infection. The broadest reasonable interpretation of the term infection merely requires that one microorganism gain entry into the cells of a host. It is known in the art that the activity of microbe X is similar to the activity of microbe Y which is known to enter the cells of a host through various pathways. Based on this similarity, it is presumed that microbe X can gain entry into the cells of a host through a multitude of avenues. There is no evidence in the specification or of record which demonstrates that preventing entry via all such avenues is credible, and therefore that utility would not be

credible and a rejection of claims 1 and 2 under 35 U.S.C. § 101/112, first paragraph, would be reasonable with respect to this utility.

Thus, the conclusion of this analysis for claim 1 is that the treatment of microbe X infection meets the criteria for a specific, substantial, and credible utility whereas the prevention of microbe X infection is not a credible utility. No rejection under 35 U.S.C. § 101 should be made against claim 1. The presence of the utility that is not credible in claim 1 (preventing microbe X infection) should be addressed in a rejection under 35 U.S.C. §112, first paragraph, scope of enablement. With respect to claim 2, both a 35 U.S.C. § 101 rejection and a 35 U.S.C. § 112, first paragraph, utility rejection should be made since the prevention utility is not credible.

Examiner's Rejection of claim 2

Claim 2 is rejected under 35 U.S.C. § 101 because the claimed invention is not supported by either a credible asserted utility or a well-established utility. Specifically, claim 2 is directed to a method of preventing microbe X infection. However, the term "infection", given its broadest reasonable interpretation consistent with the specification, merely requires that one microorganism gain entry into the cells of a host. It is known in the art that the activity of microbe X is similar to the activity of microbe Y which is known to enter the cells of a host. Based on this similarity, it is presumed that microbe X can gain entry to the cells of a host through a multitude of avenues. There is no evidence in the specification or of record which demonstrates that preventing entry via all such avenues is credible, and therefore that utility is not credible. Furthermore, since the claim is directed to a method, the utility analysis is limited to that recited

method. Claim 2 is also rejected under 35 U.S.C. § 112, first paragraph. Specifically, since the claimed invention is not supported by either a credible asserted utility or a well established utility for the reasons set forth above, one skilled in the art would not know how to use the claimed invention so that it will operate as intended without undue experimentation.

Attorney Arguments with Evidence (Alternative I)

Claim 2 has been rejected by the examiner under 35 U.S.C. § 101 and 35 U.S.C. 112, ¶1. The examiner asserts that a credible utility has not been disclosed. Reconsideration under 37 CFR 1.111 is requested.

In support of applicants' statement of utility, attached hereto is an opinion declaration under 37 CFR 1.132 by an expert in the art (see In re Alton, 76 F.3d 1168, 37 USPQ2d 1578 (Fed. Cir. 1996)) who states that it is known that microbe X only gains entry into the cells of a host through the mucosa in the nose and mouth. The expert goes on to say that administering compound A blocks the mechanism by which microbe X enters the cells of the mucosa thereby preventing infection by the microbe. The only reasonable conclusion that could be reached based on the declaration and the fact that the statements made by the examiner are unsupported by evidence to the contrary is that preventing microbe X infection is, in fact, credible. For these reasons, the utility rejections under 35 U.S.C. § 101 and 35 U.S.C. § 112, first paragraph, should be withdrawn.

Examiner's Response to Attorney Arguments with Evidence (Alternative I)

If the examiner has no additional documentation to support the argument that microbe X gains entry into the cells of a host through a multitude of avenues so as to rebut the opinion declaration, the examiner should withdraw the utility rejections.

Attorney Arguments with Evidence (Alternative II)

Claim 2 has been rejected by the examiner under 35 U.S.C. § 101 and 35 U.S.C. § 112, ¶1. The examiner asserts that a credible utility has not been disclosed. Reconsideration under 37 CFR 1.111 is requested.

In support of applicants' statement of utility, attached hereto is a factual declaration under 37 CFR 1.132 by an expert with examples that unequivocally show that microbe X only gains entry into the cells of a host through the mucosa in the nose and mouth. The declaration also demonstrates that administering compound A blocks the mechanism by which microbe X enters the cells of the mucosa thereby preventing infection by the microbe. The only reasonable conclusion that could be reached based on the declaration and the fact that the statements made by the examiner are unsupported by evidence to the contrary is that preventing microbe X infection is, in fact, credible. For these reasons, the utility rejections under 35 U.S.C. § 101 and 35 U.S.C. § 112, first paragraph, should be withdrawn.

Examiner's Response to Attorney Arguments with Evidence (Alternative II)

The examiner should withdraw the utility rejections.

Example 2: <u>Prevention</u>

Specification: The specification relates to prevention or retardation of aging by administering an effective amount of compound A.

Claims:

1. A method for preventing aging comprising administering to a patient in need thereof an effective amount of compound A.

2. A method for retarding the aging process comprising administering to a patient in need thereof an effective amount of compound A.

Analysis: The following analysis includes the questions that need to be asked according to the guidelines and the answers to those questions based on the above facts:

1. Based on the record, is there a "well established utility" for the claimed invention? Since each claim is directed to specific method of use, the utility of each claim is limited to that use and the examiner should not look to a "well established utility" for the composition used in the claimed method. Consequently, the answer to the question is no.

2. Has the applicant made any assertion of utility for the specifically claimed invention? The answer is yes, i.e., a method for preventing or retarding aging.

3. Is the asserted utility specific? The method of using compound A requires the particular application of a single particular compound to be used in the claimed method. Therefore, the utility is specific.

4. Is the asserted utility substantial? Both preventing and retarding aging clearly define a "real world" context of use and, therefore, are substantial utilities.

5. Is the asserted "specific and substantial utility" credible? Since no material has been found to date which has been shown to or would be expected to prevent or retard aging and there are no working examples or other evidence in the record which would provide credibility to these claims it would be reasonable to conclude that the utility would not be credible based on the record.

Thus, the conclusion from this analysis is that both a 35 U.S.C. § 101 rejection and a 35 U.S.C. § 112, first paragraph, utility rejection should be made.

Note, had there been an indication in the specification that applicant's invention is the treatment of symptoms associated with aging, such as skin wrinkles, then the rejection could be avoided if claims are amended to clearly state treatment of symptoms or effects of aging.

Examiner's Rejection

Claims 1 and 2 are rejected under 35 U.S.C. § 101 because the claimed invention is not supported by either a credible asserted utility or a well-established utility.

The broadest reasonable interpretation of the claims in this situation is prevention of the aging process. In this rejection it is presumed that applicants intend to prevent or retard physiological aging and not

chronological aging, since the latter reads on the stoppage of time, which is contrary to the laws of nature and therefore not credible. The preventing or retarding of aging via systemic treatment is itself not credible on its face in view of contemporary knowledge in the art. No compound is currently known which would have these effects.

Physiological aging is a multi-faceted process which does not involve a single chemical or biological effect. Various theories have been propounded (see Lehninger et al., pages 341, 344, and 886 and Scandalios, pages 40 and 41) including (1) loss of telomerase activity and the relationship of telomere length to cell death, (2) accumulation of DNA mutations, and (3) temporal genes which regulate the output of structural genes. In view of these theories, one skilled in the art would conclude that the diverse aspects of aging, e.g. loss of muscle tone, slowing of metabolism, graying of hair, etc. operate via different mechanisms. There is no reason why one skilled in the art would expect a single compound to prevent or retard all of these diverse aspects.

Heretofore the art has recognized only the topical treatment of the external manifestations of aging, e.g., skin wrinkling, as an anti-aging utility (see U.S. Patent No. 5,340,568, for example). Note that skin wrinkling is but a single manifestation of the general process of aging.

Furthermore, since the claims are directed to methods, the utility is limited to those recited methods and there is no well-established utility for such methods.

Claims 1 and 2 are also rejected under 35 U.S.C. § 112, first paragraph. Specifically, since the claimed invention is not supported by either a credible asserted utility or a well established utility for the reasons set forth above, one skilled in the art would not know how to use the claimed invention so that it would operate as intended without undue experimentation.

Attorney Arguments Only (Alternative I)

Claims 1 and 2 have been rejected by the examiner under 35 U.S.C. § 101 and 35 U.S.C. § 112, ¶1. The examiner asserts that a credible utility has not been disclosed. Reconsideration under 37 CFR 1.111 is requested.

Anti-aging is indeed a credible utility. In USP 5157031 to Schwartz et al., compounds related to dehydroepiandrosterone (DHEA) are stated as having an anti-aging utility. Long-term treatment with DHEA itself is also known to delay the rate of aging. See column 1, lines 60-64 and column 2, lines 41-42. The patented compounds exhibit the same effects of DHEA, but are more potent and produce no estrogenic effects.

This is similar to the fact pattern in In re Brana, 34 USPQ 2d 1436 (Fed. Cir. 1995). In Brana the court reversed the examiner's rejection under 35 U.S.C. § 112, ¶ 1 because the antitumor compounds at issue therein were disclosed by the applicant as superior to known antitumor agents. This *inter alia* was deemed sufficient to render credible the disclosed anti-cancer utility. See footnote 9 in Brana wherein the court notes the examiner's statement that a rejection under 35 U.S.C. § 101 for failure to disclose a practical utility also could have been made.

The examiner is also reminded that a patent is presumed valid under 35 U.S.C. 282. The examiner in the Schwartz et al. patent could have required cancellation of any utility which he deemed incredible (In re Gottlieb, 140 USPQ 665 (CCPA 1964) and Ex parte Hozumi, 3 USPQ 2d 1059 (Bd. Pat. App and Inter. 1984)), but he did not. Accordingly, one may presume that the utility disclosed in Schwartz et al. is a valid, credible utility.

Examiner's Response to Attorney Arguments Only (Alternative I)

Claims 1 and 2 are rejected under 35 U.S.C. § 101 because the claimed invention is not supported by either a credible asserted utility or a well-established utility for the reasons of record.

Claims 1 and 2 are rejected under 35 U.S.C. § 112, first paragraph. Specifically, since the claimed invention is not supported by either a credible asserted utility or a well established utility for the reasons set forth above, one skilled in the art would not know how to use the claimed invention so that it would operate as intended without undue experimentation.

Applicants' arguments have been considered, but are not deemed persuasive.

Schwartz et al. disclose anti-aging as one among many utilities, including treatment and/or prevention of cancer, obesity, diabetes, and hyperlipidemia. The claims of Schwartz et al. are process claims limited to prophylaxis of obesity only. No actual anti-aging data are disclosed in Schwartz et al. Thus this fact pattern is not analogous to the fact pattern of Brana. In In re Brana

the issue was anti-leukemic activity of compounds based on evidence obtained in art recognized models validated with analogous compounds.

Failure of the examiner in Schwartz et al. to require cancellation of the anti-aging utility does not prove that said utility is credible. Examiners do not require applicant to delete reference to utilities which are not recited in the claims and which are not specific, substantial and credible. See e.g., "Discussion of Public Comments," Final Utility Examination Guidelines, 60 FR 36263 (1995) 1177 O.G. 146 (1995).

Attorney Arguments Only (Alternative II)

Claims 1 and 2 have been rejected by the examiner under 35 U.S.C. § 101 and 35 U.S.C. § 112, ¶1. The examiner asserts that a credible utility has not been disclosed. Reconsideration under 37 CFR 1.111 is requested.

Applicants submit that even if, *arguendo*, anti-aging is not a credible utility, applicants have nevertheless satisfied the utility requirement because another utility is disclosed in the specification as filed. Example IV discloses the instant compound A when formulated for topical administration is effective in retarding the wrinkling of skin. Since only one utility is necessary to satisfy 35 U.S.C. § 101, applicants submit that the examiner's rejection is in error and should be withdrawn.

Examiner's Response to Attorney Arguments Only (Alternative II)

Claims 1 and 2 are rejected under 35 U.S.C. § 101 because the claimed invention is not supported by either a credible asserted utility or a well-established utility for the reasons of record.

Claims 1 and 2 are rejected under 35 U.S.C. § 112, first paragraph. Specifically, since the claimed invention is not supported by either a credible asserted utility or a well established utility for the reasons set forth above, one skilled in the art clearly would not know how to use the claimed invention so that it would operate as intended without undue experimentation.

Applicants' arguments have been considered, but are not deemed persuasive. Applicants are not claiming compound A. If compound A was being claimed, then any disclosed utility could be attributed thereto. However, since method of use claims are involved herein, applicants are limited to the utility set forth in those claims, i.e. retarding or preventing the entire process of aging. Claims amended so as to be drawn to a method of retarding wrinkling of skin by topical administration of compound A would obviate this rejection.

Attorney Arguments with Evidence (Alternative III)

Claims 1 and 2 have been rejected by the examiner under 35 U.S.C. § 101 and 35 U.S.C. § 112, ¶1. The examiner asserts that a credible utility has not been disclosed. Reconsideration under 37 CFR 1.111 is requested.

In support of applicants' statement of utility, attached hereto is a declaration under 37 CFR 1.132 by the inventors which shows unequivocally that the claimed compound A markedly reduces wrinkling of the skin when applied topically to the human face. The effect is long-lasting as shown in the data in Table 1.

Applicants submit that the claims encompass topical administration. Note page 20 of the specification which sets forth the various modes of administration, including topical administration. Applicants' data in the Rule 132 declaration evince a true retardation of skin wrinkling, evidence that the aging process is indeed retarded. It is not an incredible leap from retardation to prevention. One need only begin applying the material before the onset of wrinkling to lead to prevention.

Examiner's Rebuttal to Attorney Arguments with Evidence (Alternative III)

Claims 1 and 2 are rejected under 35 U.S.C. § 101 because the claimed invention is not supported by either a credible asserted utility or a well-established utility for the reasons of record.

Claims 1 and 2 are rejected under 35 U.S.C. § 112, first paragraph. Specifically, since the claimed invention is not supported by either a credible asserted utility or a well established utility for the reasons set forth above, one skilled in the art clearly would not know how to use the claimed invention so that it would operate as intended without undue experimentation.

Applicants' arguments and declaration under 37 CFR 1.132 have been considered, but are not deemed persuasive.

The claims recite retardation or prevention of aging and are given their broadest reasonable interpretation when read in light of and consistent with the specification. It is evident that retardation and/or prevention of the **entire** aging process is intended. As noted in the first Office action, skin wrinkling is but a single external manifestation of the general process of aging. One cannot conclude from applicants' data that internal organs have ceased aging because wrinkling on a test subject's face has been reduced by cosmetic application of compound A. Physiological aging is a multifaceted process which does not involve a single chemical or biological effect. This is evident in the various theories that exist, such as loss of telomerase activity and the relationship of the telomere length to cell death as well as accumulation of DNA mutations and temporal genes that regulate the output of structural genes.

Claims amended so as to be drawn to a method of retarding wrinkling of skin by topical administration of compound A would obviate this rejection.

Example 3: Therapeutic Proteins

Specification: The specification discloses a protein having the amino acid sequence of SEQ ID NO: 1 and discloses that the protein can be made by protein synthesis techniques well known in the art. The only disclosed utility for the protein is for curing Alzheimer's disease. There is no other disclosure of any chemical, physical, or biological properties of the protein. There are 98 pages of specification which disclose alternate administration techniques and dosages that are very specific, conventional techniques for protein administration. There are no working examples that demonstrate the specifically asserted utility.

Claim: 1. The isolated protein consisting of the amino acid sequence set forth in SEQ ID NO: 1.

Analysis: The following analysis includes the questions that need to be asked according to the guidelines and the answers to those questions based on the above facts:

1) Based on the record, is there a "well established utility" for the claimed invention? The specification as filed does not disclose or provide any evidence that points to an activity for the protein and furthermore there is no art of record that discloses or suggests any activity for the claimed protein. Therefore there is no well-established utility.

2) Has the applicant made any assertion of utility for the specifically claimed invention? Here, there is an asserted utility, i.e., curing Alzheimer's disease.

3) Is the asserted utility specific? Curing Alzheimer's disease, a well known disease, clearly defines a use that depends upon the particular protein disclosed. Therefore, the utility is specific.

4) Is the asserted utility "substantial"? Since a cure for Alzheimer's disease is a desirable outcome based upon a need in the art, the disclosed use of the claimed protein is substantial and "real world".

5) Is the asserted "specific and substantial utility" credible? To answer this question one must keep in mind what one skilled in the art already knows. With respect to Alzheimer's disease, one skilled in the art knows that the disease has no known cure, no known cause or mechanism, and can not even be definitively assigned as a differential diagnosis in the absence of a post mortem examination. While the specification discloses conventional protein administration techniques, it does not include any working examples. It would be reasonable to conclude that the utility would not be credible based on the evidence of record.

Thus, the conclusion that can be reached from this analysis is that both a 35 U.S.C. § 101 rejection and a 35 U.S.C. § 112, first paragraph, utility rejection should be made.

Assume for the moment that a first Office action on the merits was mailed to applicant which included utility rejections under 35 U.S.C. § 101 and § 112, first paragraph, for the reasons stated above. In response, applicant argues that while the specifically disclosed utility may not be credible, the claim is to a protein and that proteins, in view of their unique chemical structure, would have a "well established utility" as being a source of amino acids used for manufacturing supplements for vitamins or food, as protein supplements for animal food, or as an animal poison if the protein is toxic. Furthermore, it would not require undue experimentation to use the protein in any one of these manners. Thus, applicant argues, the utility rejections under 35 U.S.C. § 101 and § 112, first paragraph, are not appropriate. Such an argument should not be persuasive. A well established utility is a specific, substantial and credible utility which is well known, immediately apparent or implied by the specifications' disclosure of the properties of a material, alone or taken with the knowledge of one skilled in the art. "Well established utility" is does not mean any utility that one can dream up for an invention or a nonspecific utility that would obviously apply to virtually every member of a very general class of materials, such as proteins or DNA. If this were the case, any product or apparatus, including perpetual motion machines, would have a "well established utility" as landfill or a paper weight, any carbon containing molecule would have a "well established utility" as a fuel since it can be burned, and any protein would have the above noted well established utilities. This is not the intention of the statute.

Example 4: Uncharacterized Proteins

Specification: The specification discloses a protein having the amino acid sequence of SEQ ID NO: 1 and discloses that the protein can be made by protein synthesis techniques well known in the art. There is no disclosed utility and no description of the chemical, physical, or biological properties for the protein other than the sequence.

Claim: 1. The isolated protein consisting of the amino acid sequence set forth in SEQ ID NO: 1.

Analysis: The following analysis includes the questions that need to be asked according to the guidelines and the answers to those questions based on the above facts:

1) Based on the record, is there a "well established utility" for the claimed invention? The specification as filed does not disclose or provide any evidence that points to an activity for the protein. Additionally, there is no art of record that discloses or suggests any activity for the claimed protein. Therefore there is no well-established utility.

2) Has the applicant made any assertion of utility for the invention? No.

Thus, the conclusion of this analysis is that both a 35 U.S.C. § 101 rejection and a 35 U.S.C. § 112, first paragraph, utility rejection should be made.

Examiner's Rejection

Claim 1 is rejected under 35 U.S.C. § 101 because the claimed invention is not supported by either a specific and substantial asserted utility or a well- established utility.

The claimed protein is not supported by either a specific and substantial asserted utility or a well established utility because the specification fails to assert any utility for the protein and neither the specification as filed nor any art of record disclose or suggest any activity for the protein such that any utility would be well established for the protein.

Claim 1 is also rejected under 35 U.S.C. § 112, first paragraph. Specifically, since the claimed invention is not supported by either a specific asserted utility or a well established utility for the reasons set forth above, one skilled in the art would not know how to use the claimed invention so that it would operate as intended without undue experimentation.

Attorney Arguments

Claim 1 has been rejected by the examiner under 35 U.S.C. § 101 and 35 U.S.C. § 112, ¶1. The examiner's position is that there is neither an asserted utility nor a well-established utility for the claimed protein. Reconsideration under 37 CFR 1.111 is requested.

While the specification may not specifically assert a utility for the claimed protein, proteins as a general class of compounds have a well-established utility in view of their unique chemical structure. Specifically, because of the unique chemical structure of the claimed protein, it has a well

established utility as being a source of amino acids used for manufacturing supplements for vitamins or food, as protein supplements for animal food, or as an animal poison if the protein is toxic. Furthermore, it would not require undue experimentation to use the protein in any one of these manners. Thus, the utility rejections under 35 U.S.C. § 101 and § 112, first paragraph, are not appropriate and should be withdrawn.

Examiner's Response to Attorney Arguments Only

Claim 1 is rejected under 35 U.S.C. § 101 because the claimed invention is not supported by either a specific and substantial asserted utility or a well-established utility because of the reasons set forth in the previous Office action.

Claim 1 is also rejected under 35 U.S.C. § 112, first paragraph. Specifically, since the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility for the reasons set forth above, one skilled in the art would not know how to use the claimed invention.

Applicant's arguments have been fully considered but they are not deemed persuasive. Applicant argues that the claimed protein has a well established utility as being a source of amino acids used for manufacturing supplements for vitamins or food, as protein supplements for animal food, or as an animal poison if the protein is toxic. This is not persuasive. A "well-established utility" is a specific, substantial and credible utility which is well known, immediately apparent, or implied by the specification's disclosure of the properties of a material, alone or taken with the knowledge of one skilled

in the art. Neither a "well-established utility" nor a "specific utility" applies to any utility that one can dream up for an invention or even a utility that would apply to virtually every member of a general class of materials, such as proteins or DNA. If this were the case, any product or apparatus, including a perpetual motion machine, would have a well-established utility as landfill or a paper weight; any carbon containing molecule would have a well established utility as a fuel since it can be burned; and any protein would have the above noted "well established" or "specific" utilities. This is not the intention of the statute.

Example 5: Partially Characterized Proteins

Specification: The specification discloses a protein having the amino acid sequence of SEQ ID NO: 1 and discloses that the protein can be made by protein synthesis techniques well known in the art. There is no explicitly disclosed utility for the protein. However, there is an example which demonstrates that when the protein is contacted with whole blood, the protein will specifically bind with another protein X such that X can be isolated and quantified.

Claim: 1. The isolated protein consisting of the amino acid sequence set forth in SEQ ID NO: 1.

Analysis: The following analysis includes the questions that need to be asked according to the guidelines and the answers to those questions based on the above facts:

1) Based on the record, is there a "well established utility" for the claimed invention? Here, the specification as filed does disclose or provide evidence that points to an activity for the protein, i.e., when contacted with whole blood, it will specifically bind to protein X to enable the isolation and quantification of X. Assuming that the art does not disclose anything regarding the significance of X, or the examiner is unaware of any such art, then it would be reasonable to conclude that there is no "well established utility".

2) Has the applicant made any assertion of utility for the invention? Yes. The presence of an example may be an implicit assertion. In this case there is an implicit assertion that the claimed protein binds protein X.

3) Is the asserted utility specific? Yes. In this case the example indicates that when the protein is contacted with whole blood, the claimed protein will specifically bind to protein X.

4) Is the asserted utility substantial? No. There is no disclosed or real world utility associated with the claimed protein. Further experimentation is necessary to attribute a utility to the claimed protein. *See Brenner v. Manson*, 383 U.S. 519, 535–36, 148 USPQ 689, 696 (1966) (noting that "Congress intended that no patent be granted on a chemical compound whose sole "utility" consists of its potential role as an object of use-testing", and stated, in context of the utility requirement, that "a patent is not a hunting license. It is not a reward for the search, but compensation for its successful conclusion.").

Thus, the conclusion of this analysis is that both a 35 U.S.C. §101 rejection and a 35 U.S.C. §112 first paragraph rejection should be made.

Note: If the art disclosed at the time of filing that, e.g., an increased level of X correlates with an increased risk of heart disease, the claimed invention may have a well-established utility.

Example 6: Therapeutic Antibodies

Specification: The specification discloses a pharmaceutical composition containing a carrier, a non-antibody protein X and an antibody, said composition being suitable for treating HIV-1 infections. The specification further discloses a method of treating a subject by administering to the subject an amount of the above noted pharmaceutical composition effective to reduce the likelihood of the subject's becoming infected with HIV-1. The specification also discloses a vaccine for HIV-1 comprising the non-antibody protein X.

The specification further discloses a method of treating an HIV-infected subject, which includes administering to the subject an amount of the composition of the invention effective to reduce the rate of spread of HIV-1 infection in the subject.

The specification also discloses a method of decontaminating a fluid containing HIV-1 which comprises contacting the fluid with the composition of the invention under conditions such that the composition of the invention forms a complex with the HIV-1 therein and removing the complex so formed from the fluid, thereby decontaminating the fluid.

Claims: The following claims are pending in the application:

1. A composition comprising (a) a pharmaceutically acceptable carrier, (b) a non-antibody protein X, and (c) an antibody, said composition being suitable for treating HIV-1 infections.

2. A method of treating an HIV-1 infected subject, which comprises administering to the subject an amount of the composition of claim 1 effective to reduce the rate of spread of HIV-1 infection in the subject.

3. A method of decontaminating a fluid containing HIV-1, which comprises contacting the fluid with the composition of claim 1 under conditions such that the composition of claim 1 forms a complex with the HIV-1 therein and removing said complex from the fluid, thereby decontaminating the fluid.

4. A method of preventing a subject from becoming infected with HIV-1 comprising administering to the subject an amount of the composition of claim 1 effective to prevent the subject from becoming infected with HIV-1.

5. A method of preventing or treating HIV-1 infection which comprises administering to a subject the composition of claim 1.

6. A vaccine for HIV-1 comprising a non-antibody protein X.

Analysis: The following analysis includes the questions that need to be asked according to the guidelines and the answers to those questions based on the above facts:

1) Based on the record, is there a "well established utility" for the claimed invention? The specification as filed does not disclose or provide

any evidence that points to an activity for the compositions (claims 1 and 6) such that another non-asserted utility would be well established. Additionally, there is no art of record that discloses or suggests any activity for the claimed compositions such that another non-asserted utility would be well established. With respect to the method claims (claims 2-5), since each of these claims is directed to a specific method of use, the utility of each claim is limited to that use and the examiner should not look to a "well established utility" for the composition used in each claimed method. Consequently, the answer to the question is no for all of the claims.

2) Has the applicant made any assertion of utility for the specifically claimed invention? For each of the claims presented for this example, an asserted utility can be found. Those utilities are (1) a composition for treating HIV-1 infections (claim 1); (2) a vaccine against HIV-1 (claim 6); (3) a method of treating a subject infected with HIV-1 (claim 2); (4) a method of decontaminating a fluid containing HIV-1 (claim 3); and (5) a method of preventing a subject from becoming infected with HIV-1 (claims 4 and 5).

3) Is the asserted utility specific? HIV-1 infection is a known problem and the utilities noted in 2) above are disclosed uses that depend upon the particular protein disclosed. Therefore, the utility is specific.

4) Is the asserted utility substantial? Since all of the asserted utilities are practical based upon a need in the art, the disclosed utilities are substantial and "real world".

5) Is the asserted "specific and substantial utility" credible? The answer to that question for claims 1-3, 5 (treating part) and 6 is yes in that all of these claims are directed to subject matter which one would believe is credible. Those credible utilities are listed above in 2). However, claim 4 and part of claim 5 are directed to a method of preventing a subject from becoming infected with HIV-1. The term infection, given its broadest reasonable interpretation consistent with the specification, merely requires that one such virus gain entry into the cells of a host. Given that there are no compounds known that would be capable of preventing entry into every cell with 100% efficiency then the utility for this claim would not be credible.

Thus, the conclusion that can be reached from this analysis is that no rejection under 35 U.S.C. § 101 should be made against claims 1-3 and 6 but that both a 35 U.S.C. § 101 rejection and a 35 U.S.C. § 112, first paragraph, utility rejection should be made against claim 4. For claim 5, since only one utility is needed for the claim to meet the criterion for 35 U.S.C. § 101 and the treatment of HIV-1 infection meets this criterion, no rejection for lack of utility should be made against claim 5. The presence of the utility that is not credible in claim 5 (preventing HIV-1 infection) should be addressed under 35 U.S.C. § 112, first paragraph, scope of enablement.

Example 7: Chemical therapeutics

Specification: The specification discloses compound A where A is a stable 8-10 membered bicyclic aromatic heterocyclic having 1-3 heteroatoms selected from the group consisting of P, Se and Si. These compounds are disclosed to be useful in the inhibition of HIV protease, the prevention or treatment of infection by the human immunodeficiency virus (HIV) and the treatment of consequent pathological conditions such as AIDS. Treating AIDS or preventing or treating infection by HIV is defined as including, but not limited to, treating a wide range of states of HIV infection: AIDS, ARC (AIDS related complex), both symptomatic and asymptomatic, and actual or potential exposure to HIV. For example, the compounds of this invention are useful in treating infection by HIV after suspected past exposure to HIV by, e.g., blood transfusion, organ transplant, exchange of body fluids, bites, accidental needle stick, or exposure to patient blood during surgery.

An assay for inhibition of microbial expressed HIV protease and a cell spread assay are disclosed. Compound X, a species of the generic invention is tested in these assays.

Claims:

1. A compound of the formula:

A

where A is a stable 8-10 membered bicyclic aromatic heterocyclic having 1-3 heteroatoms selected from the group consisting of P, Se and Si.

2. A composition comprising a compound of claim 1, for use in the treatment of AIDS, in the prevention of infection by HIV, in the treatment of infection of HIV, or in the inhibition of HIV protease, and a carrier.

3. A method of treating AIDS, comprising administering to a mammal in need of such treatment an effective amount of a compound of claim 1.

4. A method of preventing infection by HIV, comprising administering to a mammal in need of such treatment an effective amount of a compound of claim 1.

5. A method of treating infection by HIV, comprising administering to a mammal in need of such treatment an effective amount of a compound of claim 1.

6. A method of inhibiting HIV protease, comprising administering to a mammal in need of such treatment an effective amount of a compound of claim 1.

7. A method of delaying the onset of AIDS, comprising administering to a mammal in need of such treatment an effective amount of a compound of claim 1.

Analysis: The following analysis includes the questions that need to be asked according to the guidelines and the answers to those questions based on the above facts:

1) Based on the record, is there a "well established utility" for the claimed invention? The specification as filed does not disclose or provide any evidence that points to an activity for the compound and composition (claims 1-2) such that another non-asserted utility would be well established. Additionally, there is no art of record that discloses or suggests any activity for the claimed compound and composition such that another non-asserted utility would be well established. Therefore the answer is no for claims 1 and 2. With respect to the method claims (claims 3-7), since each of these claims is directed to a specific method of use, the utility of each claim is limited to that use and the examiner should not look to a "well established utility" for the composition used in each claimed method.

2) Has the applicant made any assertion of utility for the specifically claimed invention? Looking at each of the claims presented for this example, you will find an asserted utility for each of them. Those utilities are (1) methods of treating AIDS or subjects infected with HIV (claims 3

and 5); (2) a method of preventing infection by HIV (claim 4); (3) a method of inhibiting HIV protease (claim 6); (4) a method for delaying the onset of AIDS (claim 7). The compound of claim 1 and the composition of claim 2 are disclosed to be useful in any of the above utilities.

3) Is the asserted utility specific? Since the claims are drawn to compound A (a relatively small genus) and various methods of using A, and because the claimed processes require the use of A, the utilities are specific.

4) Is the asserted utility substantial? Since HIV infection is a known problem, the utilities noted in 2) above clearly define a "real world" context of use and therefore are substantial utilities.

5) Is the asserted "specific and substantial utility" credible? The answer to that question for claims 1-2 (utilities other than preventing infection by HIV), 3, and 5-7 is yes in that all of these claims are directed to subject matter which one of skill in the art would believe is credible. Those credible utilities are listed above in 2). However, claim 4 is directed to a method of preventing a subject from becoming infected with HIV. The term infection, given its broadest reasonable interpretation consistent with the specification, merely requires that one such virus gain entry into the cells of a host. Given that there are no compounds known that would be capable of preventing entry into every cell with 100% efficiency then the utility for this claim would not be credible.

Thus, no rejection under 35 U.S.C. § 101 should be made against claims 3 and 5-7 but both a 35 U.S.C. § 101 rejection and a 35 U.S.C. § 112, first paragraph, utility rejection should be made against claim 4. For claim

1, since it is a product claim that does not recite any utilities, only one credible asserted utility is needed to meet the criteria for 35 U.S.C. § 101. Any of the asserted utilities, other than preventing HIV infection, meets this criteria and, accordingly, no rejection under 35 U.S.C. § 101 should be made against claim 1. For claim 2, since only one utility is needed for the claim to meet the criteria for 35 U.S.C. § 101 and the claimed utilities, other than preventing HIV infection, meet this criteria, no rejection under 35 U.S.C. § 101 should be made against claim 2. The presence of the utility that is not credible in claim 2 (preventing HIV infection) should be addressed under 35 U.S.C. § 112, first paragraph, scope of enablement.

Note that when examining the patentability of the composition of claim 2, the statement of intended use should be treated as a claim limitation when considering compliance with the requirement of 35 U.S.C. §112, first paragraph. Examination should address the issue of whether one skilled in the art could make and use the claimed invention without undue experimentation such that it would operate in the manner recited in the claim.

Example 8: "Therapeutics" Not Associated with a Disease

Specification: Compound A is disclosed to inhibit enzyme XYZ, a well-known enzyme which is a member of the family of tyrosine kinases, *in vitro*. The specification states that compound A can be used to treat diseases caused or exacerbated by increased activity of enzyme XYZ. No actual diseases are named.

Claims:

1. Compound A.

2. A method of treating a disease caused or exacerbated by increased activity of enzyme XYZ consisting of administering an effective amount of compound A to a patient.

Analysis: The following analysis includes the questions that need to be asked according to the guidelines and the answers to those questions based on the above facts:

1) Based on the record, is there a "well established utility" for the claimed invention? With respect to claim 2, since the claim is directed to a specific method of use, the utility of this claim is limited to that use and the examiner should not look to a "well established utility" for the composition used in the claimed method. Consequently, the answer to the question is no for claim 2. With respect to claim 1, the answer is different. Enzymes catalyze certain reactions involving the enzyme substrate. Here, since enzyme XYZ is a well-known tyrosine kinase, the substrate for the enzyme and the reaction which the enzyme catalyzes must also be well known.

Since all of this is well known it is reasonable to infer that an inhibitor of enzyme XYZ, such as compound A, would have a "well-established utility" in controlling the enzyme/substrate interaction in the known reaction. Therefore, compound A has a "well established utility", no rejection under 35 U.S.C. § 101 should be made against claim 1, and there is no need to go further in the analysis with respect to claim 1.

2) Has the applicant made any assertion of utility for the specifically claimed invention? The answer is yes. Claim 2 has the asserted utility of treating a disease caused or exacerbated by increased activity of enzyme XYZ.

3) Is the asserted utility specific? In this case, the specification teaches that the claimed compound inhibits a particular enzyme (XYZ). Therefore, compound A has properties and uses that are not applicable to a general class of compounds. Therefore, the answer is that the invention of claim 2 has a specific utility.

4) Is the asserted utility substantial? Since neither the specification nor the art of record disclose any diseases or conditions caused or exacerbated by enzyme XYZ, the asserted utility in this case essentially is a method of treating an unspecified, undisclosed disease or condition, which does not define a "real world" context of use. Treating an unspecified, undisclosed disease or condition would require or constitute carrying out further research to identify or reasonably confirm a "real world" context of use. Therefore, the answer to this question is no with respect to claim 2.

Therefore no rejection under 35 U.S.C. § 101 should be made against claim 1 but both a 35 U.S.C. § 101, as well as 35 U.S.C. § 112, first paragraph, utility rejection should be made against claim 2.

Once the rejection has been made with respect to claim 2, the applicant bears the burden of rebutting it. Upon receiving applicant's response, the examiner should review the original disclosure, any evidence relied upon in establishing the utility rejections under 35 U.S.C. § 101 and 35 U.S.C. § 112, first paragraph, any amendments and any new reasoning or evidence provided by the applicant in support of the asserted utility.

The following situations are most probable:

(1) Applicant provides a reference, published before the filing date of the application, which teaches that certain diseases are associated with increased activity of enzyme XYZ. In this case the examiner should withdraw the utility rejections under 35 U.S.C. § 101 and 35 U.S.C. § 112, first paragraph, for claim 2.

(2) Applicant submits an opinion declaration under 37 C.F.R. 1.132 by a qualified person of skill in the art which states that specific disease conditions are known to the skilled artisan to be either caused or exacerbated by increased activity of enzyme XYZ. The declarant identifies specific diseases and/or conditions. After reviewing the record in its entirety, the Examiner should only maintain this rejection if evidence of more probative value than the declaration exists which establishes a basis for doubting the objective truth of the declaration. Unsupported scientific reasoning is not more probative than the declaration. If the examiner maintains the rejection,

the examiner must provide documentation on the record which establishes the basis of doubting the statements made in the declaration.

(3) Applicant submits a declaration under 37 C.F.R. 1.132 which contains a factual showing that compound A is effective in alleviating the symptoms of peptic ulcers. The declaration also contains a factual showing that peptic ulcers are exacerbated by increased activity of enzyme XYZ. The facts are adequate to show that as of the date for which priority was sought, compound A was known to be effective in alleviating the symptoms of peptic ulcers. The rejection under 35 U.S.C. 101 and 112 would be withdrawn.

Example 9: DNA Fragments

Specification: The specification discloses 4332 nucleic acid sequences that were obtained from a human cDNA library that was formed using human epithelial cells. The sequences, SEQ ID NOS: 1-4332, are believed by applicant to be fragments of full length genes. Thus, the specification discloses that all of the sequences comprise at least part of the coding sequence for a protein that is actually produced in the human cells. The specification discloses how to use each of the 4332 nucleic acid sequences as a probe to obtain the full length genes that correspond to the nucleic acid sequences, which full length genes can be used to recombinantly make the corresponding proteins, which can then be used to study the cellular mechanisms and activities in which the proteins are involved. There is a generic disclosure of how to recombinantly make the corresponding protein from each of the sequences. The sequences vary in length. Some of the sequences are long enough to encode functional proteins, i.e., these sequences could be open reading frames.

No use is disclosed for any of the putative proteins other than the possibility of using them to identify and study the cellular mechanisms and activities in which the proteins are involved.

Claim 1. A cDNA consisting of the sequence set forth in SEQ ID NO: 1.

Analysis: The following analysis includes the questions that need to be asked according to the guidelines and the answers to those questions based on the above facts:

1) Based on the record, is there a "well established utility" for the claimed invention? The specification as filed does not disclose or provide any evidence that points to an activity for the cDNA or the proteins that can be obtained using the cDNA such that another non-asserted utility would be well established. Additionally, there is no art of record that discloses or provides any evidence that points to an activity for the target cDNA or the proteins that might be obtained using the target cDNA to be obtained such that another non-asserted utility would be well established. Consequently, the answer to the question is no.

2) Has the applicant made any assertion of utility for the specifically claimed invention? Here, there is an asserted utility, i.e., each cDNA can be used as a probe to obtain the full length gene that corresponds to the cDNA molecule, which full length gene can be used to recombinantly make the corresponding protein, which can then be used to study the cellular mechanisms and activities in which the protein is involved.

3) Is the asserted utility specific? The answer to this question is no. The use of the claimed nucleic acid is not particular to the sequence being claimed because it would be applicable to the general class of cDNAs. Any partial nucleic acid prepared from any cDNA may be used to as a probe in the preparation and or identification of a full-length cDNA.

4) Is the asserted utility substantial? The answer to this question is no. As seen in 2) above, the asserted utility for the claimed cDNA is a method of making the corresponding protein. Thus, to determine whether or not this method has a "substantial utility," it must be determined whether or not the corresponding protein, has a "substantial utility." Here, the only utility

asserted for the protein is for identifying and studying the properties of the protein itself or the mechanisms in which the protein is involved. This does not define a "real world" context of use. Since the asserted utility for the protein (identifying and studying the properties of the protein itself or the mechanisms in which the protein is involved) does not define a "real world" context of use, a method of making that protein also could not define a "real world" context of use. In fact, both utilities would require or constitute carrying out further research to identify or reasonably confirm a "real world" context of use.

Thus, the conclusion reached from this analysis is that both a 35 U.S.C. § 101 rejection and a 35 U.S.C. § 112, first paragraph, utility rejection should be made.

Examiner's Rejection

Claim 1 is rejected under 35 U.S.C. § 101 because the claimed invention is not supported by specific and substantial utility or a well-established utility.

The claimed cDNA compound is not supported by a specific asserted utility because the disclosed use of the nucleic acid is generally applicable to any nucleic acid and therefore is not particular the nucleic acid sequence being claimed. Further, the claimed cDNA compound is not supported by a substantial utility because the specification states only that the cDNA compounds are useful as probes for assisting in the isolation of full-length cDNAs or genes which would be used to make protein. Once the protein is obtained, the protein would be used in conducting research to functionally

characterize the protein. A starting material that can only be used to produce a final product does not have a substantial asserted utility in those instances where the final product is not supported by a specific and substantial utility. In this case none of the proteins that are to be produced as final products resulting from processes involving the claimed cDNA have asserted or identified specific and substantial utilities. The research contemplated by Applicants to characterize potential protein products, especially their biological activities, does not constitute a specific and substantial utility. Identifying and studying the properties of the protein itself or the mechanisms in which the protein is involved does not define a "real world" context of use. Note, because the claimed invention is not supported by a specific and substantial asserted utility for the reasons set forth above, credibility has not been assessed. Neither the specification as filed nor any art of record discloses or suggests any property or activity for the cDNA compounds such that another non-asserted utility would be well established for the compounds.

Claim 1 is also rejected under 35 U.S.C. § 112, first paragraph. Specifically, since the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility for the reasons set forth above, one skilled in the art would not know how to use the claimed invention.

Example 10: <u>DNA Fragment encoding a Full Open Reading Frame (ORF)</u>

Specification: The specification discloses that a cDNA library was prepared from human kidney epithelial cells and 5000 members of this library were

sequenced and open reading frames were identified. The specification discloses a Table that indicates that one member of the library having SEQ ID NO: 2 has a high level of homology to a DNA ligase. The specification teaches that this complete ORF (SEQ ID NO: 2) encodes SEQ ID NO: 3. An alignment of SEQ ID NO: 3 with known amino acid sequences of DNA ligases indicates that there is a high level of sequence conservation between the various known ligases. The overall level of sequence similarity between SEQ ID NO: 3 and the consensus sequence of the known DNA ligases that are presented in the specification reveals a similarity score of 95%. A search of the prior art confirms that SEQ ID NO: 2 has high homology to DNA Ligase encoding nucleic acids and that the next highest level of homology is to alpha-actin. However, the latter homology is only 50%. Based on the sequence homologies, the specification asserts that SEQ ID NO: 2 encodes a DNA ligase.

Claim 1: An isolated and purified nucleic acid comprising SEQ ID NO: 2.

Analysis: The following analysis includes the questions that need to be asked according to the guidelines and the answers to those questions based on the above facts:

1) Based on the record, is there a "well established utility" for the claimed invention? Based upon applicant's disclosure and the results of the PTO search, there is no reason to doubt the assertion that SEQ ID NO: 2 encodes a DNA ligase. Further, DNA ligases have a well-established use in the molecular biology art based on this class of protein's ability to ligate DNA. Consequently the answer to the question is yes.

Note that if there is a well-established utility already associated with the claimed invention, the utility need not be asserted in the specification as filed. In order to determine whether the claimed invention has a well-established utility the examiner must determine that the invention has a specific, substantial and credible utility that would have been readily apparent to one of skill in the art. In this case SEQ ID NO: 2 was shown to encode a DNA ligase that the artisan would have recognized as having a specific, substantial and credible utility based on its enzymatic activity.

Thus, the conclusion reached from this analysis is that a 35 U.S.C. § 101 rejection and a 35 U.S.C. § 112, first paragraph, utility rejection should not be made.

Example 11: <u>Animals with Uncharacterized Human Genes</u>

Specification: Kidney cells from a patient with Polycystic Kidney (PCK) Disease have been used to make a cDNA library. From this library 8000 nucleotide "fragments" have been sequenced but not yet used to express proteins in a transformed host cell nor have they been characterized in any other way. The 50 longest fragments, SEQ ID NO: 1-50, respectively, have been used to make transgenic mice. None of the 50 lines of mice have developed Polycystic Kidney Disease to date. The asserted utility is the use of the mice to research human genes from diseased human kidneys. The disease is inheritable, but chromosomal loci have not yet been identified. Neither the absence or presence of a specific protein has been identified with the disease condition.

Claims: 1. A non-human animal in which all of the somatic and germ cells contain DNA having SEQ ID NO: 1.

2. A non-human animal in which all of the somatic and germ cells contain DNA having SEQ ID NO: 2.

[3. - 50. are identical in form to 1 and 2 with the sequence number corresponding with the claim number in each.]

51. A method of screening for potential causative agents which trigger or exacerbate Polycystic Kidney Disease comprising administering a selected agent to a non-human animal of any one of claims 1 -50 and observing the kidney of said animal for abundant cyst formation.

Analysis: The following analysis includes the questions that need to be asked according to the guidelines and the answers to those questions based on the above facts:

1) Based on the record, is there a "well established utility" for the claimed invention? The specification as filed does not disclose or provide any evidence that points to a property of the claimed animals (claims 1-50) such that another non-asserted specific and substantial credible utility would be well established. Additionally, there is no art of record that discloses or provides any evidence that points to a property of the claimed animals (claims 1-50) such that another non-asserted specific and substantial credible utility would be well established. With respect to claim 51, since it is directed to a specific method of use, the utility of this claim is limited to that use and the examiner should not look to a "well established utility" for the

composition used in the claimed method. Consequently, the answer to the question is no.

2) Has the applicant made any assertion of utility for the specifically claimed invention? Here, there is an asserted utility, i.e., to use the animals to research human genes from diseased human kidneys, specifically to use the animals in a method for screening for potential causative agents which trigger or exacerbate Polycystic Kidney Disease.

3) Is the asserted utility specific? The answer to this question is yes. In this case, the sequences (claims 1-50 and the full length counterparts of the other 7950 nucleic acid fragments) are asserted to be useful to generate the non-human animals as instantly claimed, and to use the animals in a screening method for PCK.

4) Is the asserted utility substantial? The answer to this question is yes because a disease model for PCK disease is a real world context of use.

5) Is the asserted utility credible? The answer to this question is no. In this case it is noted in the specification that none of the 50 lines of mice that have been transformed with the claimed DNAs have developed Polycystic Kidney Disease to date. Additionally, there is no indication that the absence or presence of a specific protein is associated with the disease condition.

Thus, the conclusion that can be reached from this analysis is that both a 35 U.S.C. § 101 rejection and a 35 U.S.C. § 112, first paragraph, rejection should be made.

Examiner's Rejection

Claims 1-51 are rejected under 35 U.S.C. § 101 because the claimed invention is not supported by either a credible asserted utility or a well-established utility.

Neither the specification as filed nor any art of record discloses or suggests any specific property or activity for the animals such that a utility would be well established for the animals.

Further, the claimed animals and method of screening are not supported by a credible utility because the specification states that none of the transgenic animals exhibited PCK disease. The asserted use of the animals is for research in human genes from diseased kidneys however the specification indicates that they were unable to get an operative model. Since there is no evidence on the record that there are operative transgenic animal models for this research, the asserted utility is inoperative and is therefore not credible.

With regard to the asserted use of the animals as disease models, the action of the human DNA compounds on the animals is not specifically known and the mere assertion that abundant cyst formation will be observable in any of the claimed animals would not be accepted by one skilled in the art as being reasonable or credible in view of the contemporary knowledge in the art. As discussed by A. Cure et al. (a 1995 reference), while extensive studies have been conducted, the only clear results are from Mendelian studies of families that exhibit the disease. These studies indicate that the disease is inheritable and dominant, as opposed to recessive, via statistical analysis. No study has clearly indicated that a single DNA component is involved. No chromosomal loci have been identified. The

possibility of a regulatory mechanism being involved has not been ruled out by any of the studies conducted to date. No specific protein or abnormal level of a specific protein has been associated with the disease. The expectation that any of the claimed animals will exhibit the abundant cyst formation based on the presence of a single, unidentified DNA compound is not credible based on the specification's evidence to the contrary.

Claims 1-51 are also rejected under 35 U.S.C. § 112, first paragraph. Specifically, since the claimed invention is not supported by either a credible asserted utility or a well established utility for the reasons set forth above, one skilled in the art would not know how to use the claimed invention so that it would operate as intended without undue experimentation.

Attorney Arguments Only (Alternative I)

Claims 1-51 have been rejected by the examiner under 35 U.S.C. § 101 and 35 U.S.C. § 112, ¶1. The examiner asserts that a substantial utility has not been disclosed. Reconsideration under 37 CFR 1.111 is requested.

The use of these animals to study DNA and polycystic kidney disease via observing abundant cyst formation is credible. This utility is directly analogous to that of US Patent No. 4,736,866 to Leder et al. in which human DNA compounds associated with tumor formation are contained in the genomes of non-human animals and these animals are used to study the human DNA compounds and tumor formation as well as tumor treatment. Such an important medical research utility as exists for the current claimed invention is a patentable utility. The claimed animals contain DNA

compounds that are associated with human cells which exhibit the specific disease, just as they were in the Leder et al. patent.

Examiner's Response to Attorney Arguments Only (Alternative I)

Claims 1-51 are rejected under 35 U.S.C. § 101 because the claimed invention is not supported by either a credible asserted utility, or a well established utility for the reasons of record.

Claims 1-51 are rejected under 35 U.S.C. § 112, first paragraph. Specifically, since the claimed invention is not supported by either a credible asserted utility, or a well established utility for the reasons set forth above, one skilled in the art would not know how to use the invention so that it would operate as intended without undue experimentation.

Applicants' arguments have been considered, but are not deemed persuasive. Applicants analogize the current specification, animals and intended utilities to those of Leder et al. US Patent No. 4,736,866. The situations are in fact not analogous. The specific embodiment of the specific MYC oncogene in the Leder et al. patent involved a well-established oncogene. There was no question in the art that the particular DNA compound had been directly associated with tumor formation in humans. Moreover, the specific mice disclosed in the Leder et al. specification exhibit tumor formation. It does not directly follow that a diseased cell will necessarily contain "culprit" DNA as asserted by Applicants. This is particularly true of cDNA compounds as used herein, where no protein effect is associated with the disease, nor are there any operative animal models that exhibit this disease state and the evidence of record is contrary

to the desired result. Thus, even if one were to accept the premise that the diseased cell must contain a genetic flaw, no transgenic model is disclosed in currently available form.

Attorney Arguments with Evidence (Alternative II)

Claims 1-51 have been rejected by the examiner under 35 U.S.C. § 101 and 35 U.S.C. § 112, ¶1. The examiner asserts that a credible utility has not been disclosed. Reconsideration under 37 CFR 1.111 is requested.

In support of applicant's statement of utility, attached hereto is a declaration submitted under 37 CFR 1.132 by the inventors which describes a mouse corresponding to the animal of claim 38 which has exhibited abundant cyst formation. This effect has been confirmed as evidenced in the declaration, by the production of three additional founder mice that carry DNA SEQ ID NO: 38 as a transgene and have exhibited abundant cyst formation. In addition, as evidenced in the declaration, these mice have been cross-bred and some of their progeny exhibit the abundant cyst formation as well.

Based on this evidence clearly the use of the claimed animals to screen for agents which trigger or exacerbate the disease condition is substantial and credible.

Examiner's Response to Attorney Arguments with Evidence (Alternative II)

The examiner should withdraw the rejection of claim 38 based on lack of credible utility in light of this evidence. However, the other product

claims should still be rejected under 35 U.S.C. §101 and 35 U.S.C. §112 first paragraph as lacking credible utility and claim 51 should still be rejected under 35 U.S.C. §112 first paragraph as lacking an enabling disclosure except as it depends on claim 38.

Example 12: Receptors

Specification: The specification discloses a protein, isolated from a cell membrane preparation, which is the binding partner for protein X. The specification does not characterize the isolated protein with regard to its biological function or any disease or body condition that is associated with the isolated protein. Based solely on the fact that the protein was isolated from a cell membrane and it binds to protein X, applicant characterizes the isolated protein as receptor A. The function of protein X has also not been identified. The specification discloses a binding assay for determining other materials which bind to the receptor by adding the material to the complex of receptor A and protein X and determining the amount of inhibition of the binding of the complex as an indication that the material will bind to the receptor and thus be a therapeutic drug to effect control over the receptor. Also disclosed is the production of a monoclonal antibody that specifically binds to receptor A. There are no working examples using any materials to demonstrate such inhibition of binding, to assay the receptor or to identify any other material which binds to the receptor. The utility disclosed is for identifying materials that bind the receptor and the potential use of such materials as therapeutics.

Claims:

1. Isolated receptor A.

2. A method of identifying materials which bind to receptor A comprising:

a) forming a complex of receptor A and protein X in a liquid;

b) adding a material to be screened to said complex;

c) determining the amount of binding of said complex wherein an inhibition of said binding is an indication that said material binds to said receptor.

3. A monoclonal antibody which specifically binds to receptor A.

Analysis: The following analysis includes the questions that need to be asked according to the guidelines and the answers to those questions based on the above facts. For this fact situation, each claim will be analyzed separately.

Claim 1:

1) Based on the record, is there a "well established utility" for the claimed invention? The specification as filed does not disclose or provide any evidence that points to a property of the claimed receptor such that another non-asserted utility would be well established. Additionally, there is no art of record that discloses or provides any evidence that points to a property of the claimed receptor such that another non-asserted utility would be well established. Consequently, the answer to the question is no.

2) Has the applicant made any assertion of utility for the specifically claimed invention? Here, there is an asserted utility for the claimed invention. In fact, for claim 1 there are two asserted utilities, i.e., a) a method of identifying materials which bind to receptor A, and b) a method of making a monoclonal antibody.

3) Is the asserted utility specific? The answer to this question is yes. In this case, the method of identifying materials which bind to a specific receptor, namely receptor A and a method of making monoclonal antibodies to receptor A are methods that are not applicable to the general class of receptors. Therefore, there is an asserted specific utility for the claimed invention.

4) Is the asserted utility substantial? The answer to this question in each case is no. The method in 2a) above is a method of identifying those materials which bind to receptor A. Thus, to determine whether or not this method has a "substantial utility," it must be determined whether or not the material that binds to receptor A itself has a "specific and substantial utility." Here, the only utility asserted for the identified materials is a therapeutic to effect control over receptor A. Since neither the specification nor the art of record disclose any diseases or conditions associated with receptor A, a method of treating an unspecified, undisclosed disease or condition, does not define a "real world" context of use. Further research to identify or reasonably confirm a "real world" context of use is required. Since the asserted utility for the identified materials does not define a "real world" context of use, a method of identifying such materials also could not define a "real world" context of use.

The method in 2b) above is a method of making a material, i.e., a monoclonal antibody. Thus, to determine whether or not this method has a "substantial utility", it must be determined whether or not the monoclonal antibody itself has a "specific and substantial utility." Here, there is an asserted utility for the monoclonal antibody even though it is not explicit,

e.g., as a therapeutic drug to effect control over the receptor. However, since neither the specification nor the art of record disclose any diseases or conditions associated with receptor A, the asserted utility in this case essentially is a method of treating an unspecified, undisclosed disease or condition, which does not define a "real world" context of use. Treating an unspecified, undisclosed disease or condition clearly would require or constitute carrying out further research to identify or reasonably confirm a "real world" context of use. *See Brenner v. Manson*, 383 U.S. 519, 535–36, 148 USPQ 689, 696 (1966) (noting that "Congress intended that no patent be granted on a chemical compound whose sole "utility" consists of its potential role as an object of use-testing", and stated, in context of the utility requirement, that "a patent is not a hunting license. It is not a reward for the search, but compensation for its successful conclusion.").

Since the asserted utility for the product (monoclonal antibody) does not define a "real world" context of use, a method of making such a product also could not define a "real world" context of use.

Thus, the conclusion from analysis is that both a 35 U.S.C. § 101 rejection and a 35 U.S.C. § 112, first paragraph, utility rejection should be made on claim 1.

Claim 2:

1) Based on the record, is there a "well established utility" for the claimed invention? Since the claim is directed to a specific method of use, the utility of this claim is limited to that use and the examiner should not

look to a "well established utility" for the composition used in the claimed method. Consequently, there is no "well-established" utility for the method.

2) Has the applicant made any assertion of utility for the specifically claimed invention? Here, there is an asserted utility for the claimed invention, i.e., a method of identifying materials that bind to receptor A.

3) Is the asserted utility specific? The answer to this question is yes. In this case, the method of identifying materials which bind to a specific receptor, namely receptor A, is a method that is not applicable to the general class of receptors. It is specific to receptor A. Therefore, there is an asserted specific utility for the claimed invention.

4) Is the asserted utility substantial? The answer to this question is no. Specifically, the method essentially is a method of identifying a material, i.e., those materials which bind to receptor A. Thus, to determine whether or not this method has a "substantial utility", it must be determined whether or not the material that binds to receptor A itself has a "substantial utility." Here, the only utility asserted for the identified materials is a therapeutic to effect control over receptor A. Since neither the specification nor the art of record disclose any diseases or conditions associated with receptor A, the asserted utility in this case essentially is a method of treating an unspecified, undisclosed disease or condition, which does not define a "real world" context of use. Treating an unspecified, undisclosed disease or condition clearly would require or constitute carrying out further research to identify or reasonably confirm a "real world" context of use. *See Brenner v. Manson*, 383 U.S. 519, 535–36, 148 USPQ 689, 696 (1966) (noting that "Congress intended that no patent be granted on a chemical compound whose sole

"utility" consists of its potential role as an object of use-testing", and stated, in context of the utility requirement, that "a patent is not a hunting license. It is not a reward for the search, but compensation for its successful conclusion.").

Since the asserted utility for the identified materials does not define a "real world" context of use, a method of identifying such materials also could not define a "real world" context of use.

Thus, the conclusion is that both a 35 U.S.C. § 101 rejection and a 35 U.S.C. § 112, first paragraph, utility rejection should be made on claim 2.

Claim 3:

1) Based on the record, is there a "well established utility" for the claimed invention? The specification as filed does not disclose or provide any evidence that points to a property of the claimed monoclonal antibody such that another non-asserted utility would be well established. Additionally, there is no art of record that discloses or provides any evidence that points to a property of the claimed monoclonal antibody such that another non-asserted utility would be well established. Consequently, the answer to the question is no.

2) Has applicant made any assertion of utility for the specifically claimed invention? Here, there is no explicitly asserted utility for the claimed monoclonal antibody. However, as stated in the analysis of claim 1 above, there is an implied asserted utility for the monoclonal antibody even though it is not explicit, e.g., as a therapeutic drug to effect control over the receptor.

3) Is the asserted utility specific? The answer to this question is yes. In this case, the monoclonal antibody is specific for a specific protein, namely receptor A. Therefore, there is an asserted specific utility for the claimed invention.

4) Is the asserted utility substantial? The answer to this question is no. Specifically, since neither the specification nor the art of record disclose any diseases or conditions associated with receptor A, the asserted utility in this case is a method of treating an unspecified, undisclosed disease or condition, which does not define a "real world" context of use. Treating an unspecified, undisclosed disease or condition would require or constitute carrying out further research to identify or reasonably confirm a "real world" context of use. *See Brenner v. Manson*, 383 U.S. 519, 535–36, 148 USPQ 689, 696 (1966) (noting that "Congress intended that no patent be granted on a chemical compound whose sole "utility" consists of its potential role as an object of use-testing", and stated, in context of the utility requirement, that "a patent is not a hunting license. It is not a reward for the search, but compensation for its successful conclusion.").

Thus, both a 35 U.S.C. § 101 rejection and a 35 U.S.C. § 112, first paragraph, utility rejection should be made on claim 3.

Caveat:

Let us assume for the moment that the specification also discloses that receptor A is present on the cell membranes of melanoma cells but not on the cell membranes of normal skin cells. Assume also that the examiner has found and made of record a journal article published prior to the

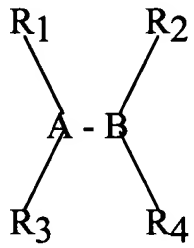
application's filing date indicating that it is desirable to selectively detect melanoma cells as opposed to normal skin cells so as to diagnose that type of cancer. Does this change the above analysis?

For each of the claims, the above analysis changes right from the first question: Based on the record, is there a "well established utility" for the claimed invention? The answer to this question would change to yes in each case. Specifically, based on this record, there is a "well established utility" for the products of claims 1 and 3. The "well established utility" for the receptor A is a method of assaying for materials that bind to receptor A by contacting the materials to a complex of receptor A and protein X. Furthermore, making a monoclonal antibody to receptor A for diagnosing melanoma would constitute a well-established utility. Such utilities are "well established" because the disclosure of the properties of the receptor and antibody taken together with the knowledge of one skilled in the art indicates that these specific, substantial and credible utilities were known. With respect to claim 2, since there is now evidence of record providing a correlation between this method and diagnosing melanoma, i.e., materials identified by the method, such as the monoclonal antibody, can be used to diagnose melanoma, this method now has a "well established utility".

Therefore, utility rejections under 35 U.S.C § 101 rejection and a 35 U.S.C. § 112, first paragraph, should not be made against claims 1-3.

Example 13: <u>Large Chemical Groups</u>

Specification: The specification discloses a genus of chemical compounds having the formula:

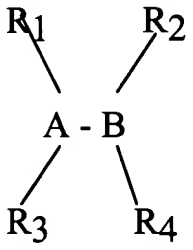


Wherein A, B and R1-R4 are defined.

The specification teaches the chemical synthesis methods necessary to make the compounds but does not disclose any chemically similar compounds. The specification provides several paragraphs describing basic experimental methods with known materials and suggests testing the claimed compounds in the same methods so as to ascertain the physical, chemical and biological properties of the claimed compounds. The only utility mentioned in the specification is that the compounds could be used for biomedical research once the physical, chemical and biological properties of the compounds have been determined.

Claims:

1. Compounds having the formula:



Analysis: The following analysis includes the questions that need to be asked according to the guidelines and the answers to those questions based on the above facts:

1) Based on the record, is there a "well established utility" for the claimed invention? The specification as filed does not disclose or provide any evidence that points to a property or activity of the claimed compounds such that another non-asserted utility would be well established. Additionally, the art of record does not disclose or provide any evidence that points to a property or activity of the claimed compounds such that another non-asserted utility would be well established. Consequently, the answer to the question is no.

2) Has the applicant made any assertion of utility for the specifically claimed invention? Here, there is an asserted utility, i.e., the claimed compounds can be used in biomedical research once the physical, chemical and biological properties of the compounds have been determined.

3) Is the asserted utility specific? The answer to this question is no. Any chemical compound can be used for biomedical research and experimental methods. This type of assertion is generic to the class of chemical compounds and therefore not specific to the claimed invention.

4) Is the asserted utility substantial? The answer to this question would be no. Biomedical research and even experimental methods for determining the physical, chemical, and biological properties of the compounds themselves do not define a "real world" context of use. Such utilities clearly would require or constitute carrying out further research to identify or reasonably confirm a "real world" context in which the compounds could be used. *See Brenner v. Manson*, 383 U.S. 519, 535–36, 148 USPQ 689, 696 (1966) (noting that "Congress intended that no patent be granted on a chemical compound whose sole utility consists of its potential role as an object of use-testing", and stated, in context of the utility requirement, that "a patent is not a hunting license. It is not a reward for the search, but compensation for its successful conclusion.").

Thus, both a 35 U.S.C. § 101 rejection and a 35 U.S.C. § 112, first paragraph, utility rejection should be made.

Examiner's Rejection

Claim 1 is rejected under 35 U.S.C. § 101 because the claimed invention is not supported by either a specific and substantial asserted utility or a well- established utility. The claimed compounds are not supported by either a specific and substantial asserted utility or a well established utility because the specification states only that the compounds are useful for

biomedical research, and neither the specification as filed nor any art of record discloses or suggests any property or activity for the compounds such that another non-asserted utility would be well established for the compounds. The biomedical research contemplated by applicants is unspecified. It will take place at some future time, only when the properties of the claimed compounds might have been elucidated by the experimental methods disclosed in applicants' specification. Absent a disclosure of those properties, the asserted utility of biomedical research lacks specificity. Note, because the claimed invention is not supported by a specific and substantial asserted utility for the reasons set forth above, credibility has not been assessed.

Claim 1 is also rejected under 35 U.S.C. § 112, first paragraph. Specifically, since the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility for the reasons set forth above, one skilled in the art would not know how to use the claimed invention so that it would operate as intended without undue experimentation.

nature immunology

SIGIRR, a negative regulator of Toll-like receptor–interleukin 1 receptor signaling

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The Toll-like receptor–interleukin 1 receptor signaling (TLR–IL-1R) receptor superfamily is important in differentially recognizing pathogen products and eliciting appropriate immune responses. These receptors alter gene expression, mainly through the activation of nuclear factor-κB and activating protein 1. SIGIRR (single immunoglobulin IL-1R-related molecule), a member of this family that does not activate these factors, instead negatively modulates immune responses. Inflammation is enhanced in SIGIRR-deficient mice, as shown by their enhanced chemokine induction after IL-1 injection and reduced threshold for lethal endotoxin challenge. Cells from SIGIRR-deficient mice showed enhanced activation in response to either IL-1 or certain Toll ligands. Finally, biochemical analysis indicated that SIGIRR binds to the TLR–IL-1R signaling components in a ligand-dependent way. Our data show that SIGIRR functions as a biologically important modulator of TLR–IL-1R signaling.

Members of the TLR–IL-1R superfamily, defined by the presence of an intracellular Toll–IL-1R (TIR) domain, are important in mediating immune responses. This superfamily can be divided into two main subgroups, based on extracellular domains: the immunoglobulin domain–containing receptors, and the leucine-rich-repeat motif–containing receptors. The immunoglobulin domain subgroup includes IL-1R1, IL-1RII (although it lacks a TIR domain), IL-18R and ST2 (also known as T1)[1]. IL-1 signals through IL-1R1 to mediate inflammatory responses, whereas IL-1RII serves as a negative regulator of IL-1 signaling by binding IL-1 (ref. 2). IL-18 promotes T helper type 1 (T_H1) cell differentiation and natural killer cell activation, whereas ST2 is important in developing T_H2 cell responses[3,4]. The leucine-rich-repeat subgroup consists of at least ten human TLRs, which are important in the recognition of pathogens[5–10].

Except for the IL-1RII and ST2 molecules, all of the well characterized members of the TLR–IL-1R superfamily serve as positive regulators of inflammation. These molecules signal through similar, although not identical, signaling pathways to activate the transcription factors nuclear factor (NF)-κB and activating protein 1 (AP-1)[11]. For IL-1-dependent signaling, the pathway is initiated when IL-1 binds to IL-1R1; the affinity of this binding is enhanced by IL-1R accessory protein. Next, the adaptor proteins MyD88 and Tollip are recruited to the receptor complex through their TIR domains[12–14]. IRAK (IL-1R-associated kinase) and IRAK4 are also recruited to the receptor complex, where IRAK becomes phosphorylated after activation and then interacts with the adaptor protein TRAF6 (tumor necrosis factor (TNF) receptor–associated factor 6; refs. 15,16). IRAK4-IRAK-TRAF6 then forms a complex with another adapter protein, Pellino 1, and leaves the receptor to interact with TAK1 (transforming growth factor-β–activated kinase 1, a member of the mitogen-activated protein kinase kinase kinase family), TAB1 (TAK1-binding protein 1) and TAB2 (TAK1-binding protein 2) on the membrane[17]. The TAK1-TAB1-TAB2-TRAF6 complex is then released to the cytosol, where TAK1 is phosphorylated and IKK (IκB kinase) is activated[18,19]. IKK phosphorylates IκB, which is prebound to NF-κB in the cytoplasm, holding NF-κB in an inactive state. After IκB is phosphorylated and ubiquitinated, it is degraded, and NF-κB is then free to enter the nucleus[20,21]. Activated TAK1 has also been linked to the activation of Jnk (Jun N-terminal kinase), which activates the c-Jun subunit of AP-1. Several TLR–IL-1Rs also use variations of this signaling pathway. For example, TLR4 and TLR3 use a MyD88-independent pathway[22–24].

Although the immune system is designed to be protective, if left unchecked, excessive or inappropriate activation of immune cells or cytokines can lead to severe inflammatory disease, such as inflammatory bowel disease, arthritis and bacterial sepsis[25–27]. The positive function of the TLR–IL-1R superfamily in inflammation has been studied extensively, but very little is known about how these pathways are negatively regulated. IRAK-M, a protein that functions as a positive regulator of NF-κB in cell lines after overexpression, functions as a negative regulator of TLR signaling in macrophages[28]. Macrophages from IRAK-M-null mice show enhanced cytokine production and activation of signaling intermediates after treatment with several different Toll ligands[28]. In addition to IRAK-M, MyD88s, an alternatively spliced form of MyD88 that blocks recruitment of IRAK-4, has also been shown to act as a negative regulator of Toll and IL-1 signaling[29,30].

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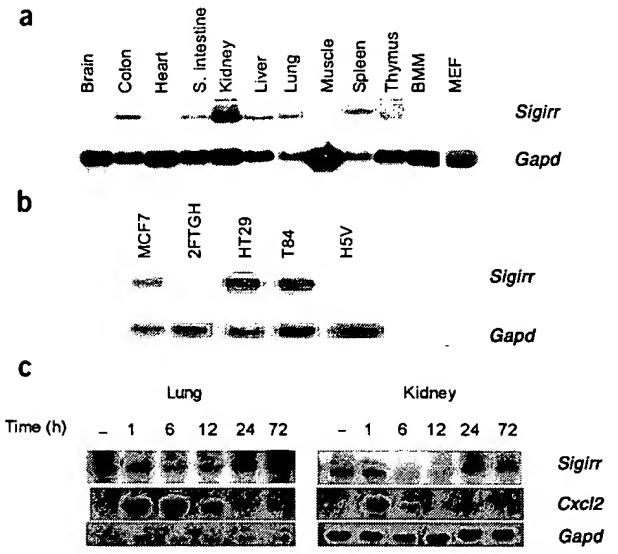

Figure 1 The expression of SIGIRR is cell- and tissue-specific and is down-regulated after immune challenge. (a) RNA from tissues, bone marrow–derived macrophages (BMM), mouse embryonic fibroblast (MEFs) and H5V cells were analyzed for SIGIRR expression by RNA blot. S., small. (b) Total RNA from the MCF7, 2FTGH, HT29, T84 and H5V cell lines was analyzed by RNA blot. (c) BALB/c mice were injected intraperitoneally with 50 μg LPS, and RNA was extracted (times, above blots) and analyzed by RNA blot. Probes (right margins): *Sigirr*, SIGIRR; *Gapd*, glyceraldehyde phosphodehydrogenase; *Cxcl2*, MIP-2.

Through expressed sequence tag database searching, we found a TIR domain–containing receptor, SIGIRR[31]. This receptor is the only TIR domain–containing receptor identified that has a single immunoglobulin domain. Although most members of this superfamily can activate NF-κB or AP-1 constitutively or after structural modification, no activation has been noted with SIGIRR. In addition, although SIGIRR has an immunoglobulin domain, it does not bind to IL-1 or enhance IL-1-dependent signaling[31]. Here we report that mice lacking SIGIRR are hyperresponsive to both endotoxin challenge and injection of IL-1. Furthermore, primary kidney cells and splenocytes from these mice show enhanced activation in response to TLR–IL-1R ligands. These results demonstrate that SIGIRR acts as a negative regulator of IL-1 and lipopolysaccharide (LPS) signaling.

RESULTS

SIGIRR is down-regulated after induction of inflammation

SIGIRR contains a single immunoglobulin domain and a highly conserved TIR domain. Unlike the rest of the well characterized TLR–IL-1Rs, no constitutive signaling, as measured by NF-κB or JNK activation, has been noted from the SIGIRR receptor either by simple overexpression or after modification of the SIGIRR structure. Although SIGIRR has a highly conserved TIR domain, it does not retain two amino acids (Ser447 and Tyr536) from IL-1R that have been shown to be essential for signaling[31].

To assess the biological function of SIGIRR, we analyzed its expression pattern. RNA blot analyses in the mouse showed SIGIRR was highly expressed in the kidney; moderately expressed in the colon, small intestine, lung, spleen and liver; and weakly or not expressed in the brain and muscle (Fig. 1a). Although the tissue expression of SIGIRR seemed ubiquitous, its cell-type expression was more specific, with extremely high expression in epithelial cells lines, such as HT29 and T84; moderate expression in splenocytes; and low or undetectable expression in fibroblast and endothelial cell lines such as 2FTGH, MEF and H5V (Fig. 1a,b and data not shown). We did not find any expression of SIGIRR in bone marrow–derived macrophages by RNA blot analysis (Fig. 1a,b).

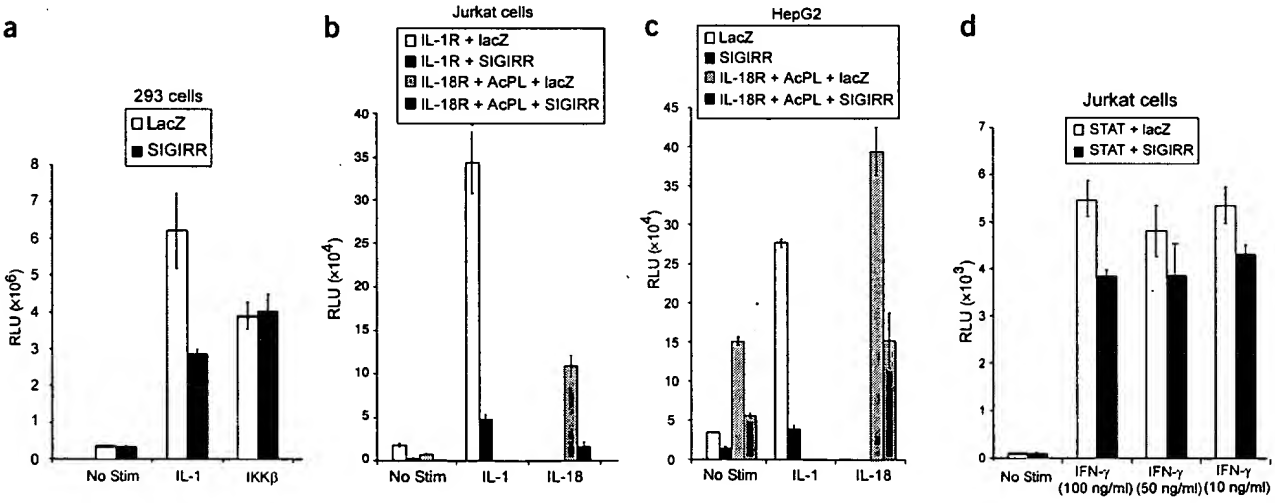

Figure 2 Overexpression of SIGIRR inhibits IL-1 and IL-18 signaling. (a) 293 cells that stably overexpress IL-1R were transfected with an NF-κB reporter as well as constructs for lacZ, SIGIRR or IKKβ. (b) Jurkat cells were transfected with an NF-κB-dependent reporter construct as well as expression constructs for lacZ, SIGIRR, IL-1R, IL-18R or AcPL. (c) HepG2 cells were transfected with an NF-κB-dependent reporter construct as well as expression constructs for lacZ, SIGIRR, IL-18R or AcPL. (d) Jurkat cells were transfected with a STAT1-dependent reporter construct as well as expression constructs for lacZ or SIGIRR. At 20 h after transfection, cells were left untreated (No stim) or were stimulated for 5 h with IL-1 (2 ng/ml; a–c), IL-18 (5 ng/ml; a–c) or IFN-γ (d; concentrations, horizontal axis). Luciferase activities were determined and expressed as relative light units (RLU). AcPL, accessory-protein-like (a subunit of IL-18R). Data are from one of three independent experiments with similar results.

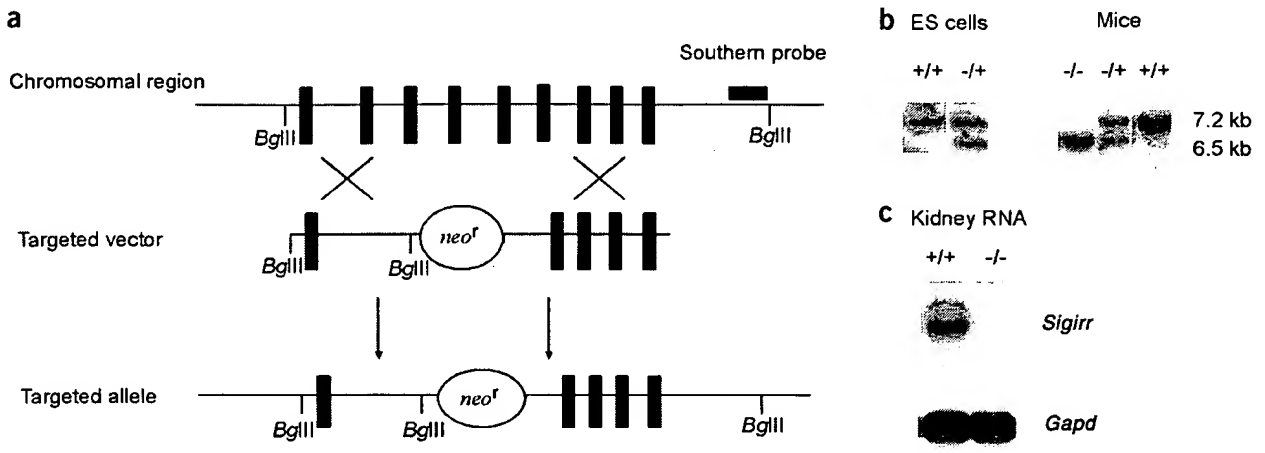

Figure 3 Targeted disruption of the mouse gene encoding SIGIRR. (**a**) The targeting vector (middle) was constructed based on the structure of the gene encoding SIGIRR (top). The predicted disrupted gene (bottom) and the location of the Southern probe (top right) are shown. Boxes, coding exons. *neo*^{r}, neomycin-resistance gene. (**b**) Southern blot analysis of DNA from SIGIRR-deficient mice and ES cells. Genomic DNA was extracted from mouse tail tissue or ES cells, digested with *Bgl*II and analyzed by Southern blot, using the probe shown in a. There was a single 7.2-kb band for the wild-type sample (+/+) and a 6.5-kb band for the homozygous sample (–/–); both bands were present for the heterozygous sample (+/–). (**c**) RNA blot analysis of SIGIRR-deficient mice. Total RNA extracted from the kidney was analyzed by RNA blot using a full-length mouse cDNA as the probe (right margins: *Sigirr*, SIGIRR; *Gapd*, glyceraldehyde phosphodehydrogenase).

As many genes that are involved in the immune response undergo changes in expression pattern after induction of inflammation, we examined SIGIRR expression in mice after injecting them with LPS. SIGIRR was down-regulated at 6 and 12 h after injection of a low dose of LPS in many tissues, including the lung and kidney (Fig. 1c). The RNA expression returned to baseline by 24 h, after mice recovered from the LPS challenge. Expression of the chemokine macrophage inflammatory protein 2 (MIP-2) indicated active inflammation in these tissues. The change in expression of SIGIRR after inflammation indicates that it may be involved in this process.

Overexpression of SIGIRR inhibits IL-1 and IL-18 signaling

To examine the potential function of SIGIRR in the IL-1 signaling pathway, we overexpressed SIGIRR in Jurkat and HepG2 cells. Overexpression of SIGIRR substantially reduced the IL-1- and IL-18-mediated activation of NF-κB, as measured by an NF-κB-dependent luciferase reporter assay (Fig. 2a–c). In contrast, SIGIRR overexpression had no effect on the interferon-γ (IFN-γ)-mediated activation of STAT1 in Jurkat cells or IKKβ overexpression in 293 cells (Fig. 2a,d). These results indicate that SIGIRR may function as a negative regulator of IL-1 and IL-18 signaling.

In vivo challenges of SIGIRR-deficient mice

To assess the function of SIGIRR in the immune response, we generated SIGIRR-deficient mice. We disrupted the gene encoding SIGIRR by homologous recombination in MC-50 embryonic stem (ES) cells. We designed a targeting vector to replace exons 2–5 and to insert stop codons in all three reading frames (Fig. 3a). A SIGIRR-targeted ES cell clone microinjected into mouse blastocysts successfully transmitted the disrupted gene encoding SIGIRR through the germline (Fig. 3b). By RNA blot analysis, we did not find SIGIRR RNA in the kidneys of SIGIRR-deficient mice (Fig. 3c). SIGIRR-deficient mice were born at the expected mendelian ratios. The mice were healthy and showed no obvious abnormalities.

As other TLR–IL-1R-deficient mice do not show obvious defects until challenged with an inflammatory stimulus, we injected mice intraperitoneally with the TLR4 activator LPS. SIGIRR-deficient mice showed a more potent inflammatory response than did wild-type

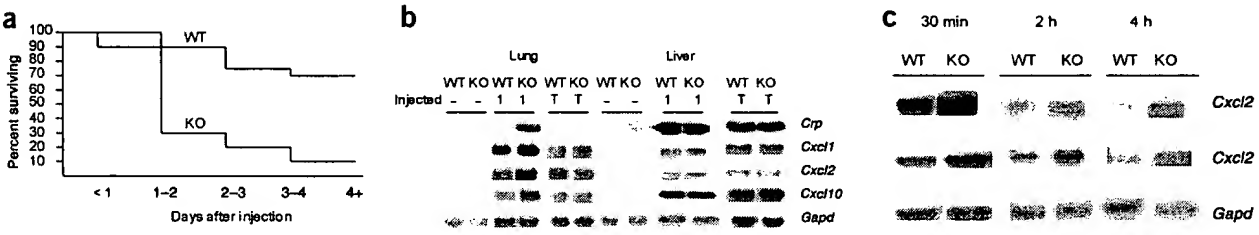

Figure 4 SIGIRR-deficient mice show enhanced inflammatory responses to LPS and IL-1. (**a**) SIGIRR-deficient mice have a reduced threshold to lethal endotoxin challenge. Wild- type (WT; $n = 12$) and SIGIRR-deficient (KO; $n = 10$) mice were injected intraperitoneally with 600 μg LPS and monitored for survival for 7 d. (**b**) SIGIRR-deficient mice show greater induction of CRP and chemokine genes. Mice were injected intraperitoneally with 250 ng IL-1 or 200 ng TNF. Mice were killed after 2 h, and RNA from lung (left) and liver (right) was analyzed by RNA blot. Data are representative of results obtained with four pairs of mice. –, no injection; 1, IL-1; T, TNF. (**c**) Enhanced chemokine expression in SIGIRR-deficient mice occurs over a long time. Mice were injected with IL-1 as described in a and killed (times, above blot), then RNA was prepared from the colon and analyzed by RNA blot. WT, wild-type; KO, SIGIRR-deficient. Probes (right margins, b,c): *Crp*, CRP; *Cxcl1*, KC; *Cxcl2*, MIP-2; *Cxcl10*, IP-10; *Gapd*, glyceraldehyde phosphodehydrogenase.

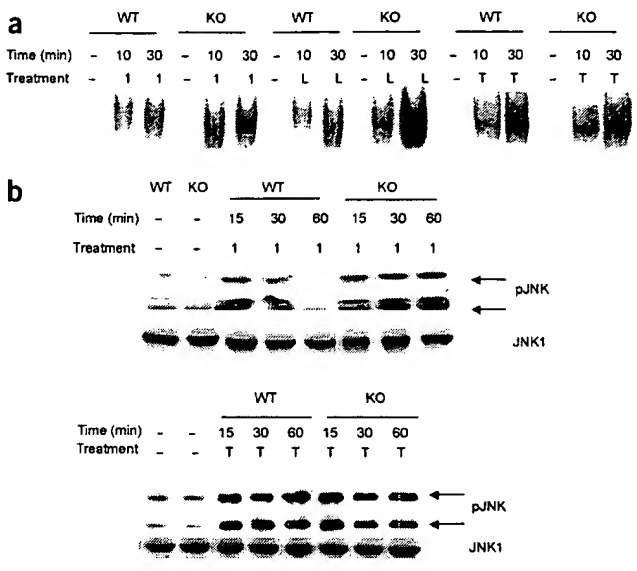

Figure 5 SIGIRR-deficient kidney cells show enhanced activation in response to LPS or IL-1. (**a**) SIGIRR-deficient kidney cells show a more profound NF-κB gel shift after IL-1 or LPS treatment. Primary mouse kidney cells were either left untreated or treated (times, above blot) with IL-1 (20 ng/ml), LPS (10 μg/ml) or TNF (20 ng/ml). The cells were then lysed and analyzed by electrophoretic mobility-shift assay with an NF-κB-specific probe. (**b**) SIGIRR-deficient kidney cells show a prolonged activation of JNK after IL-1 treatment. Cells were treated with IL-1 or TNF (times, above blot), lysed and analyzed by immunoblot with a phospho-specific JNK antibody (pJNK) and a JNK1 antibody. The two bands (arrows) shown are the two isoforms of pJNK (p46 and p54). Data are from three experiments. –, no treatment; 1, IL-1; L, LPS; T, TNF; WT, wild-type; KO, SIGIRR-deficient.

mice, as evidenced by their considerably reduced threshold to lethal endotoxin challenge (Fig. 4a). These mice succumbed more rapidly than did wild-type control mice. Whereas only 10% of the SIGIRR-deficient mice survived, 70% of the wild-type mice survived the challenge. Most SIGIRR-deficient mice succumbed to the LPS challenge between 1 and 2 d after injection, whereas almost all of the wild-type mice were alive at that time.

To determine if the negative regulatory activity of SIGIRR was specific to LPS challenge, we injected SIGIRR-deficient mice intraperitoneally with IL-1 or TNF. There was much more induction of the chemokines KC (CXC ligand 1), MIP-2 and IFN-inducible protein 10 (IP-10) in the lung, but not the liver, of SIGIRR-deficient mice than in wild-type control mice 2 h after injection of IL-1, but not TNF (Fig. 4b). Furthermore, up-regulation of acute-phase C-reactive protein (CRP) was considerably greater in the lungs of SIGIRR-deficient mice than in the lungs of wild-type mice. The basal amounts of these chemokines and CRP were very low in both wild-type and SIGIRR-deficient mice. Chemokine induction after IL-1 injection was enhanced for a longer time in SIGIRR-deficient mice than in wild-type control mice. For example, induction of the expression of chemokines KC and MIP-2 30 min, 2 h and 4 h after injection was greater in the colons of SIGIRR-deficient mice than in colons of wild-type mice (Fig. 4c). In addition, although the induction of KC and MIP-2 was relatively weak 8 h after injection, these chemokines were still more strongly expressed in SIGIRR-deficient mice than in wild-type mice (data not shown). The fact that the SIGIRR-deficient mice were hyperresponsive to both IL-1 and LPS strongly indicates that SIGIRR can function *in vivo* as a negative regulator of IL-1 and LPS signaling.

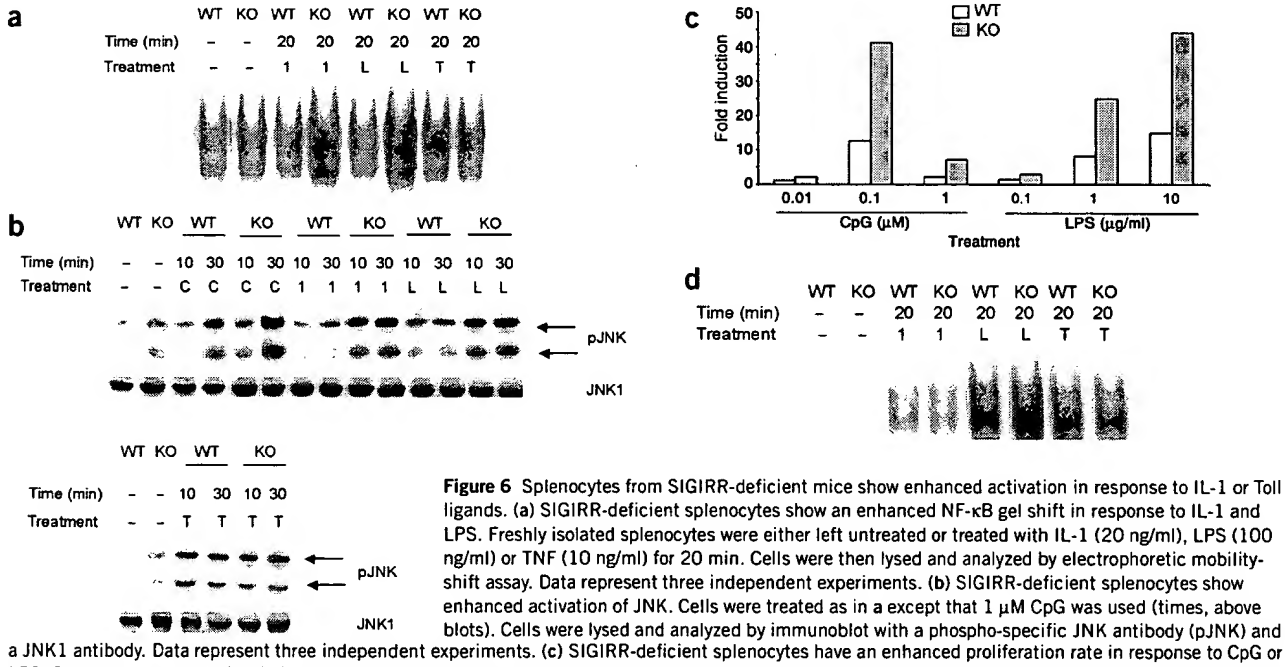

Figure 6 Splenocytes from SIGIRR-deficient mice show enhanced activation in response to IL-1 or Toll ligands. (**a**) SIGIRR-deficient splenocytes show an enhanced NF-κB gel shift in response to IL-1 and LPS. Freshly isolated splenocytes were either left untreated or treated with IL-1 (20 ng/ml), LPS (100 ng/ml) or TNF (10 ng/ml) for 20 min. Cells were then lysed and analyzed by electrophoretic mobility-shift assay. Data represent three independent experiments. (**b**) SIGIRR-deficient splenocytes show enhanced activation of JNK. Cells were treated as in a except that 1 μM CpG was used (times, above blots). Cells were lysed and analyzed by immunoblot with a phospho-specific JNK antibody (pJNK) and a JNK1 antibody. Data represent three independent experiments. (**c**) SIGIRR-deficient splenocytes have an enhanced proliferation rate in response to CpG or LPS. Splenocytes cultured in triplicate in 96-well plates were either left untreated or treated for 48 h with CpG or LPS (doses, horizontal axis). The cells were then pulsed with 1 μCi [*methyl*-^{3}H]thymidine for 12 h. Results are expressed as 'fold' induction of treated over untreated. Data represent three independent experiments. (**d**) SIGIRR-deficient and wild-type macrophages show similar NF-κB gel shift after treatment with IL-1, LPS and TNF. Bone marrow–derived macrophages were either left untreated or treated with IL-1 (20 ng/ml), LPS (10 μg/ml) or TNF (10 ng/ml) for 20 min. The cells were then lysed and analyzed by electrophoretic mobility-shift assay with an NF-κB-specific probe. –, no treatment; 1, IL-1; L, LPS; C, CpG; T, TNF; WT, wild-type; KO, SIGIRR-deficient.

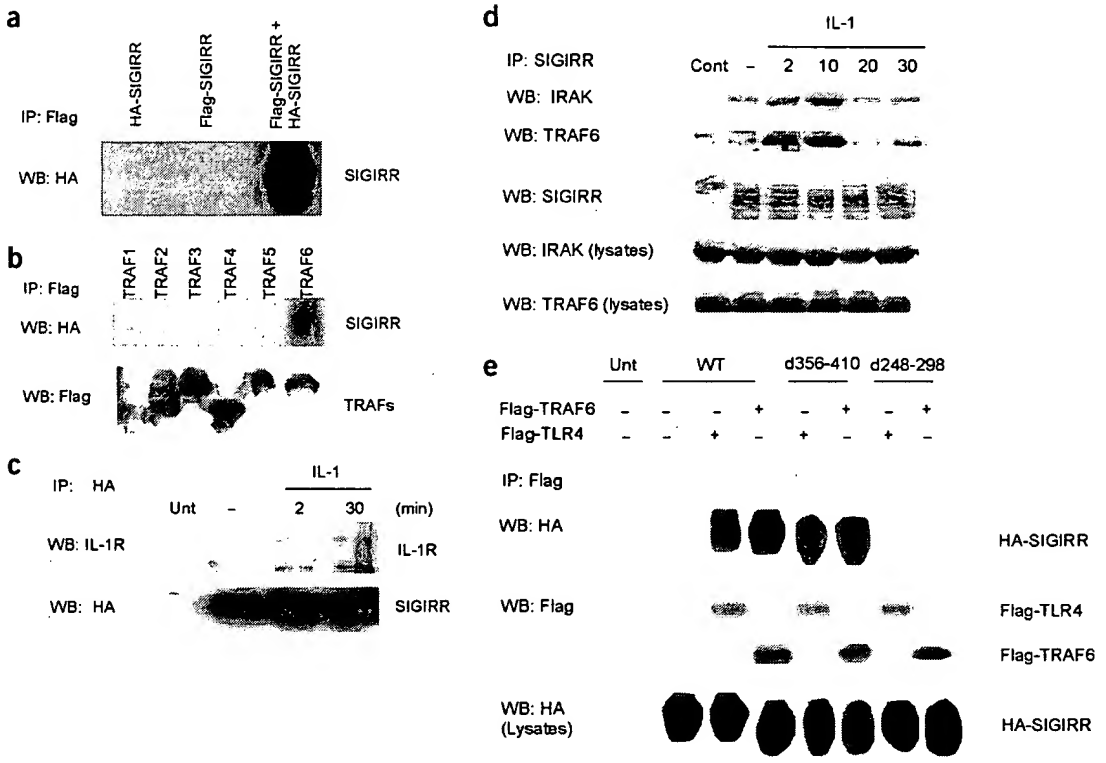

Figure 7 SIGIRR interacts with molecules involved in TLR–IL-1R signaling. (**a**) 293 cells were transfected with expression constructs for HA-SIGIRR, Flag-SIGIRR or both. At 48 h after transfection, cell lysates were immunoprecipitated with a Flag antibody, then the immunoprecipitates were analyzed by immunoblot with an HA antibody. (**b**) SIGIRR interacts specifically with TRAF6, but not TRAF1–TRAF5. 293 cells were transfected with HA-SIGIRR and a TRAF construct (above blot). At 48 h after transfection, cell lysates were immunoprecipitated with a Flag antibody, then the immunoprecipitates were analyzed by immunoblot with HA antibody or Flag antibody. (**c**) SIGIRR interacts with IL-1R in a signal-dependent way. HT29 cells stably expressing HA-SIGIRR were treated with 20 ng/ml IL-1 (times, above blot). Cell lysates were immunoprecipitated with an HA antibody, then the immunoprecipitates were analyzed by immunoblot with IL-1R antibody or HA antibody. Unt, untransfected cells. (**d**) Endogenous SIGIRR interacts with IRAK and TRAF6 in a signal-dependent manner. HT29 cells were treated with 20 ng/ml IL-1 (times, above blot). Cells lysates were immunoprecipitated with a SIGIRR antibody, then the immunoprecipitates were analyzed by immunoblot with an IRAK, TRAF6 or SIGIRR antibody. Lysates were also probed with IRAK and TRAF6, as a control. Cont, immunoprecipitated with pre-immune serum. (**e**) SIGIRR interacts with TLR4. 293 cells stably expressing wild-type (WT) HA-SIGIRR, the deletion mutant d248–298 HA-SIGIRR or the deletion mutant d356–410 HA-SIGIRR were transfected with expression constructs for Flag-TRAF6 or Flag-TLR4 (above blot; –, not transfected; +, transfected). At 48 h after transfection, cell lysates were immunoprecipitated with a Flag antibody, then the immunoprecipitates were analyzed by immunoblot with an HA antibody and a Flag antibody. Lysates were probed with an HA antibody to demonstrate expression of the SIGIRR constructs. Unt, untransfected cells. SIGIRR protein appears as a smear after SDS-PAGE because of its heavy glycosylation (data not shown). IP, immunoprecipitation; WB, immunoblot.

Ex vivo challenges of SIGIRR-deficient cells

To determine if SIGIRR is involved in immediate signaling events through the TLR–IL-1R family, we examined several primary cell types from SIGIRR-deficient mice. Because SIGIRR is highly expressed in mouse kidney, we isolated primary kidney epithelial cells that were found to strongly express SIGIRR (data not shown). The SIGIRR-deficient cells had enhanced NF-κB DNA-binding activity in response to IL-1 and LPS but not TNF at both 10 and 30 min after stimulation (Fig. 5a). These cells also had a prolonged activation of JNK in response to IL-1. Although the peak JNK phosphorylation occurred at 15 min in the wild-type cells, the peak was maintained for up to 1 h after IL-1 stimulation in the SIGIRR-deficient cells (Fig. 5b).

Like the kidney cells, SIGIRR-deficient splenocytes also showed considerably enhanced NF-κB and JNK activation in response to LPS or IL-1, but not TNF (Fig. 6a,b). Moreover, SIGIRR-deficient splenocytes showed a two- to threefold-higher rate of proliferation after treatment with LPS or CpG, further indicating that these cells were hyperresponsive to LPS and CpG (Fig. 6c). The results with different SIGIRR-deficient primary cells are consistent with the *in vivo* studies in SIGIRR-deficient mice described above, demonstrating that SIGIRR has a negative regulatory function in the signaling pathways mediated by IL-1 and LPS, and probably has a similar function in the CpG-TLR9 pathway.

As a control, we found no difference in the ligand-induced activation of downstream kinases, NF-κB DNA binding, or gene expression between wild-type and SIGIRR-deficient mice in bone marrow–derived macrophages, in which SIGIRR expression was not detectable (Fig. 6d and data not shown). Therefore, the negative regulatory function of SIGIRR is likely to be cell-type specific, given its differential expression in different cell types.

Interactions of SIGIRR with other signaling molecules

To elucidate the mechanism of action of SIGIRR, we assessed its interactions with known components of the TLR–IL-1R pathway. In coimmunoprecipitation experiments in 293 cells, SIGIRR, like many other

TLRs, was strongly dimerized with itself and interacted with several members of the TLR–IL-1R superfamily, such as TLR4, TLR5 and TLR9, but most strongly with the IL-1R (Fig. 7 and data not shown). SIGIRR also interacted specifically with the adaptor protein TRAF6, but not TRAF1-5 adaptor proteins not involved in TLR–IL-1R pathways (Fig. 7b). There was IL-1-dependent interaction between SIGIRR and the endogenous IL-1R in HT29 cells that stably overexpressed SIGIRR (Fig. 7c). Endogenous SIGIRR also interacted in an IL-1-dependent way with endogenous IRAK and TRAF6. This induced interaction was transient, as it was maximal 2 and 10 min after treatment and then returned to basal levels (Fig. 7d). The similar time course of interaction of IRAK and TRAF6 is consistent with the fact that these two molecules form a complex after IL-1 treatment[32]. The endogenous interaction of SIGIRR with IRAK and TRAF6 after IL-1 treatment indicates that SIGIRR may exert its negative regulatory effects through these signaling components.

In addition to interacting with IL-1R and the common downstream signaling components, SIGIRR can also interact with the LPS receptor TLR4 (Fig. 7e). Coimmunoprecipitation experiments in 293 cells with SIGIRR deletion mutants showed that a portion of the TIR domain of SIGIRR (amino acids 248–298) was essential for the interaction of SIGIRR with both TLR4 and TRAF6 (Fig. 7e). In contrast, a different deletion mutant (amino acids 356–410 deleted) and wild-type SIGIRR interacted with both TRAF6 and TLR4. These results indicate that the interactions of SIGIRR with both TLR4 and TRAF6 are specific.

DISCUSSION

Although most members of the TLR–IL-1R superfamily function as positive regulators of signaling, no signaling activity has been found to emanate from the SIGIRR receptor[31]. The SIGIRR-deficient mouse demonstrates that SIGIRR acts as a negative regulator *in vivo* in response to LPS or IL-1 challenge. In particular, there was considerably reduced survival after endotoxin challenge for SIGIRR-deficient mice. Septic shock is initiated by LPS released from microorganisms during infection, and results in massive production of mediators such as the cytokines IL-1 and TNF. These mediators lead to multiple organ system failure and death. Despite extensive research, and development of many therapeutic approaches, the mortality rate from septic shock is still 30–50% (ref. 25). As SIGIRR can function as a negative regulator of LPS-mediated lethality, elucidating the mechanism of action of SIGIRR may provide a potential therapeutic approach to control the excessive inflammatory response associated with sepsis.

The regulation of SIGIRR RNA may help explain the biological function of SIGIRR as a negative regulator. Down-regulation of SIGIRR in certain inflammatory conditions may facilitate a more potent immune response. Another negative regulator of IL-1 signaling, IL-1RII, is also down-regulated after inflammatory challenge. IL-1RII down-regulation after LPS treatment in monocytes has been hypothesized to be necessary for maximum immune response[33,34].

In addition to functioning in the LPS response, SIGIRR-deficient mice also showed an enhanced inflammatory response to IL-1, as evidenced by increased chemokine expression in the lung and colon, but not the liver. This tissue specificity of the effects of SIGIRR probably results from its pattern of cell-type- and tissue-specific expression. SIGIRR is highly expressed in many epithelial cell lines and moderately expressed in splenocytes, but is not expressed in primary macrophages, fibroblasts and endothelial cells. The high expression of SIGIRR in epithelial cells indicates that SIGIRR may serve mainly to dampen the immune response in cells that are continually exposed to microorganisms, such as colon and lung epithelial cells. The similar induction of chemokines in the livers of wild-type and SIGIRR-deficient mice after IL-1 injection may be a result of either the induction of secondary cytokines or the high expression of chemokines from a non-SIGIRR-expressing cell population in the liver. The fact that SIGIRR expression is down-regulated in the liver after LPS injection in mice (data not shown) and that overexpression of SIGIRR in the liver cell line HepG2 inhibited IL-1 and IL-18 signaling indicate SIGIRR may have functional involvement in the liver. Consistent with the expression pattern of SIGIRR in different cell types, SIGIRR-deficient kidney cells and splenocytes, but not macrophages, show enhanced responsiveness to IL-1 and Toll ligands.

SIGIRR exerts its negative regulatory function in TLR–IL-1R-mediated pathways through its direct effect on the immediate signaling events, including signal-induced NF-κB and JNK activation. However, details of the mechanism by which SIGIRR exerts its effects on signaling are not yet clear. As SIGIRR interacts with IL-1R1, IRAK and TRAF6 after IL-1 treatment, SIGIRR may negatively regulate the IL-1 pathway through its interaction with the IL-1R complex. After IL-1 stimulation, receptor-proximal signaling components, including IRAK and TRAF6, are recruited to IL-1R to form a receptor complex. After their appropriate activation at the receptor complex, these signaling molecules are released from the receptor to interact and activate downstream components. SIGIRR may negatively regulate the IL-1 pathway by interfering with the appropriate recruitment and activation of the receptor-proximal signaling components (such as IRAK and TRAF6). Alternatively, SIGIRR may attenuate the dissociation of the activated signaling components from the receptor, inhibiting the activation of downstream signaling events. SIGIRR also is involved in negatively regulating LPS signaling both *in vivo* and *in vitro*. As SIGIRR can specifically interact with TLR4 and TRAF6, LPS signaling may also be inhibited by either blocking the recruitment and activation of signaling molecules or their dissociation from the receptor complex.

IRAK-M negatively regulates IL-1–TLR signaling by inhibiting the dissociation of the receptor-proximal signaling complex[28]. As IRAK-M has been described as functioning mainly in macrophages, and SIGIRR is not detected in this cell type, it is not likely that these proteins cooperate *in vivo*. However, it is still possible that both of these proteins may be induced in certain conditions and cooperate as negative regulators. Future research is warranted to examine both the mechanism of action of SIGIRR and the specific cell types in which SIGIRR exerts its effects. Because members of the TLR–IL-1R superfamily have essential functions in the innate immune response, understanding the negative regulation of these pathways is crucial in furthering our understanding of how to control inflammation.

METHODS

Biological reagents and cell culture. Recombinant human IL-1 was provided by the National Cancer Institute. Recombinant human IL-18 and IFN-γ and mouse TNF were obtained from Peprotech. LPS from *Escherichia coli* serotype O11:B4 was purchased from Sigma. The CpG oligonucleotide (5′-TCCATGACGTTCCTGACGTT-3′) was synthesized in a phosphorothioate-modified form by Invitrogen. The 293 cells stably expressing the IL-1R and HT29 cells were maintained in DMEM supplemented with 10% FCS, penicillin G (100 μg/ml) and streptomycin (100 μg/ml). The Jurkat E6.1 and HepG2 cell lines were maintained in RPMI 1640 medium supplemented with 10% FBS, glutamine and antibiotics.

Constructs and antibodies. Expression constructs for TRAF1–TRAF6 were provided by H. Wesche at Tularik (South San Francisco, California). DNA encoding hemagglutinin (HA)-SIGIRR was amplified by PCR using the following primers: 5′ primer, 5′-GAATTCGAGCCATGCCAGGTGTCTGTGATAGG-3′, and 3′ primer, 5′GAATTCAGCGTAATCTGGAACATCGTATGGGTACATATCA TCCTTGGACACCAG-3′. The I.M.A.G.E Consortium Clone 2821373 was the template; it was digested with *Eco*RI and ligated into the vector pLXIN

(Clontech). The HA-SIGIRR deletion mutants d248–298 and d356–410 (deletion of amino acids 248–298 and 356–410, respectively) were generated by PCR. DNA encoding full-length human IL-1R, IL-18R and IL-1R accessory-protein-like (a subunit of IL-18R) was subcloned into pDC304, a close relative of pDC302 (ref. 35). The *lacZ* plasmid encodes β-galactosidase driven by the cytomegalovirus promoter subcloned into the pDC304 vector. The NF-κB-dependent luciferase reporter plasmid contains three NF-κB sites fused to luciferase and has been described[36]. The signal transducer and activator of transcription 1 (STAT1)-dependent reporter plasmid (a gift from D. Smith, Amgen, Seattle, Washington) contains four direct repeats of the STAT-binding site from the Fc-γ receptor type I promoter followed by a minimal herpes simplex virus thymidine kinase promoter, and was subcloned upstream of luciferase into pGL2-basic (Promega). Expression vectors encoding human IKKβ and human TLR4 were provided by Tularik and R. Medzhitov (Yale University), respectively. Antibodies to HA (07-054; Upstate Biotechnology), SIGIRR (AF990; R&D Systems), TRAF6 (sc-7221; Santa Cruz Biotechnologies), IRAK (sc-7883; Santa Cruz Biotechnologies), IL-1R (sc-993; Santa Cruz Biotechnologies), M2-Flag (F3165; Sigma), phosphorylated JNK (9251; Cell Signaling Technology) and JNK1 (sc-474; Santa Cruz Biotechnologies) were used.

Reporter assays. Jurkat E6.1 cells (1×10^6) or HepG2 cells (4×10^5) were transiently transfected using FuGENE 6 (Roche Diagnostics), following the manufacturer's protocol. Cells were transfected with reporter plasmid (200 ng), receptor (400 ng), SIGIRR (400 ng) or *lacZ*, with a 1:3 ratio of DNA:FuGENE 6. The 293 cells stably expressing IL-1R were transfected by the calcium-phosphate method. The 293 cells were transfected with reporter plasmid (200 ng), IKKβ (100 ng) or SIGIRR (400 ng). Transfection of *lacZ* was used to ensure all samples received equal amounts of DNA. At 20 h after transfection, cells were stimulated with cytokines for 4 h (293 cells), 5 h (Jurkat cells) or 6 h (HepG2 cells). Cells were lysed and luciferase activity was assessed using Reporter lysis buffer (for Jurkat and 293 cells) or Passive lysis buffer (for HepG2 cells) and Luciferase Assay Reagent (Promega). All results reported represent duplicate experiments with at least three independent transfections.

Generation of SIGIRR-deficient mice. The SIGIRR genomic clone was obtained from screening of a C57/BL6 BAC library. The gene encoding SIGIRR was subcloned into the pGEX-KG vector and characterized by restriction analysis and DNA sequencing. A targeting vector was created to replace exons 2–5 with the neomycin-resistance gene under control of the mouse phosphoglycerate kinase 1 gene promoter. For the construction of the SIGIRR gene-targeting vector, the 5′ arm (consisting of a 1.5-kilobase (kb) fragment containing exon 1 and part of exon 2 and stop codons inserted in all three reading frames) as well as the 3′ arm (consisting of ~4 kb containing exons 5–9) were cloned into pKSNT. The herpes simplex virus thymidine kinase gene driven by the phosphoglycerate kinase 1 promoter was at the 3′ end of the targeting vector. DNA was linearized by digestion with *Asn*I and was then electroporated into MC-50 ES cells, followed by selection of G418- and gancyclovir-resistant ES cell clones. The clones of double-resistant cells were analyzed by Southern blot with a probe located outside the targeting construct. Targeted ES cells were injected into mouse blastocysts to produce chimeric mice. The chimeric mice were bred to BALB/c mice to generate wild-type, heterozygous and homozygous mice. SIGIRR-deficient mice and their age-matched wild-type littermates from these intercrosses were used for experiments. The Cleveland Clinic Foundation Animal Research Committee approved all of the animal protocols used in this study.

Transfections and primary cell isolation. HT29 cells expressing HA-SIGIRR were generated by retroviral infection. Viral supernatant suspensions were obtained by transfection of approximately 2×10^6 Phoenix cells per 60-mm dish with 5 μg HA-SIGIRR DNA cloned into pLXIN. Supernatant suspensions containing the recombinant retroviruses were incubated with the cells in medium containing 4 μg/ml Polybrene (Sigma). At 24 h after infection, the supernatant suspension was removed and cells were 'selected' in 350 μg/ml G418. The calcium-phosphate method was used to transfect 293 cells. For preparation of primary kidney cells, kidneys were cut into small pieces and incubated in 0.5% trypsin (Invitrogen) for 30 min at 37 °C. Cells from each kidney were plated in a 15-cm plate and grown to confluence for 7–10 d in DMEM supplemented with 10% FBS. The cells were then split 24 h before treatement and subjected to NF-κB gel-shift assay and immunoblot analysis. Bone marrow–derived macrophages were obtained from tibia and femur bone marrow by flushing with DMEM. The cells were cultured in DMEM supplemented with 20% FBS and 30% L929 supernatant for 5 d.

Southern and RNA blots. For Southern blot analysis, genomic DNA was extracted from ES cells or mouse tail tissue, digested with *Bgl*II, separated by 1% agarose gel electrophoresis and analyzed with a 3′ external probe. For RNA analysis, total RNA was isolated using TRIzol reageant (Invitrogen) according to the manufacturer's instruction, fractionated on a formaldehyde gel and probed with ^{32}P-labeled gene-specific DNA probes, according to the protocols provided by Amersham Biosciences.

Coimmunoprecipitations. Cells (untreated or treated) were lysed in Triton-containing lysis buffer as described[17]. Cell extracts were incubated with 1 μg antibody overnight at 4 °C with 20 μl protein A– or protein G–Sepharose beads (pre-washed and resuspended in PBS at a ratio of 1:1). After incubation, beads were washed four times with lysis buffer, separated by SDS-PAGE, transferred to Immobilon-P membranes (Millipore) and analyzed by immunoblot.

Gel-shift assay. An NF-κB-binding site (5′-GAGCAGAGGGAAATTCCGTA ACTT-3′) from the gene encoding IP-10 was used as a probe[17]. The binding reaction was done at room temperature for 20 min in a total volume of 20 μl containing 20 mM HEPES buffer, 10 mM KCl, pH 7.0, 0.1% Nonidet-P40, 0.5 mM DTT, 0.25 mM phenylmethanesulfonyl flouoride and 10% glycerol.

Splenocyte proliferation. Mouse spleens were excised and the cells were filtered through a nylon cell strainer with holes 100 μm in diameter. Cells were cultured in triplicate test and control wells at a density of 2×10^5 cells per well in a total volume of 200 μl in flat-bottomed 96-well microtiter plates. After 48 h of treatment, wells were pulsed with [*methyl*-^{3}H]thymidine (1.0 μCi/well) for 12 h. Cultures were collected by aspiration onto glass fiber filters, and incorporated radioactivity was measured by scintillation spectrometry.

Injections. Mice were injected intraperitoneally with 600 μg LPS and monitored for survival for 7 d. In other esperiments, mice were injected intraperitoneally with 250 ng IL-1 or 200 ng TNF, then tissues were dissected and homogenized in Trizol, and total RNA was prepared.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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concentration has been an enduring biological question. Answers probably lie in tissue-specific regulatory molecules, and in this respect the emergence of calcipressins as regulators of calcineurin function is a promising development. This demonstration of the regulatory function of calcipressins in T lymphocytes advances our knowledge of the immune system, adding a subtlety to calcineurin function. The phenotype of Csp1 deficiency in T cells shows the potential involvement that calcineurin regulatory proteins have in the therapeutic manipulation of the immune system.

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SIGIRR puts the brakes on Toll-like receptors

Luke AJ O'Neill

Members of the Toll-like receptor–interleukin 1 receptor superfamily signal inflammatory responses. However, a member of this family is now shown to modulate these responses by acting as a negative regulator.

The initial phase of host defense against invading microbes involves a family of proteins called Toll-like receptors (TLRs). These proteins are expressed on various cell types, most notably dendritic cells, where they act as primary sensors of microbial products and activate signaling pathways that lead to the induction of immune and inflammatory genes. TLRs belong to a broader family of proteins, which include receptors for the pro-inflammatory cytokines interleukin 1 (IL-1) and IL-18 (ref. 1). Among the best-characterized TLRs are TLR4, TLR5 and TLR9, which sense lipopolysaccharide (LPS), flagellin and CpG motifs, respectively. Although these receptors have important functions in host defense, their unrestrained stimulation may be detrimental to the host. Thus, negative regulators of IL-1 receptor (IL-1R), IL-18R and TLRs may be required to modulate their responses. In this issue of *Nature Immunology*, Wald *et al.*[2] describe an intriguing inhibitor of this receptor superfamily.

All members of the TLR–IL-1R superfamily signal inflammation in a very similar way. This is because they all contain a conserved protein sequence in their cytosolic regions, called the Toll–IL-1R (TIR) domain, which activates common signaling pathways, most notably those leading to the activation of the transcription factor NF-κB and stress-activated protein kinases. However, Wald *et al.* show that an orphan receptor, which has the rather cumbersome but accurate name, single immunoglobulin IL-1R–related protein (SIGIRR)[3], is an inhibitory member of this receptor superfamily. SIGIRR seems to temper cellular activation by IL-1, LPS and probably other activators of receptors in the TLR–IL-1R superfamily, such that the biological outcome will be the result of a balance between activation by a receptor and dampening by SIGIRR. SIGIRR therefore acts as a 'brake' on the TLR system, which may be essential for regulating the detrimental effects of innate immunity, as occurs in sepsis and chronic inflammation.

The TLR–IL-1R superfamily can be divided into three subgroups[1]. The first contains extracellular immunoglobulin (Ig) domains and includes IL-1RI. The second is the TLRs, which lack Ig domains, but have extracellular leucine-rich repeats; recent years have seen tremendous progress in determining their function. The third subgroup consists of upstream adapter molecules, including MyD88, MyD88 adapter-like (Mal, also known as TIRAP) and TIR domain–containing adaptor–inducing interferon-β (IFN-β; TRIF, also known as TICAM-1). These adapters are recruited to receptor TIR domains and initiate signalling processes through IL-1R–associated kinases (of which there are four) and the adaptor molecule TRAF-6, which leads to activation of four protein kinase cascades, culminating in the activation of NF-κB and kinases p38, JNK and p42/p44 MAP kinase[1]. These molecules in turn promote the production of many proinflammatory proteins and enhance immune reactivity. Recent evidence indicates differences in adapter usage, such that although almost all the receptors recruit MyD88, only some receptors use Mal and TRIF, leading to specificity in outcome[4]. The best example is TRIF usage by TLR3 and TLR4, which leads to the activation of IFN-regulatory factor 3 and the induction of IFN-β[5,6]. To some extent, because there has been much more progress made in the understanding of the TLR and adapter subgroups, the Ig subgroup has been neglected. Most members in this subgroup remain as orphan receptors of unknown function, the exceptions being IL-1R and IL-18R and their respective accessory proteins, and IL-1Rrp2, which may be a receptor for IL-1F9, a 'paralog' of IL-1 (ref. 7). Five other IL-1 paralogs occur in humans and it seems likely that they will be ligands or antagonists for the orphan receptors[8]. Defining a function for SIGIRR therefore assigns a function to one of the orphans; it is of considerable interest that this function is inhibitory for other members of the protein superfamily.

Wald *et al.* show that SIGIRR is expressed in various mouse tissues, including epithelial cells in the kidney, and is highly expressed in the colon but less so in spleen cells. Bone marrow–derived macrophages did not express SIGIRR. Because LPS down-regulates SIGIRR expression in epithelial cells, this indicates that SIGIRR might be inhibitory, consistent with the fact that SIGIRR has a TIR domain that lacks two amino acids essential for signaling by IL-1RI. To test this possibility, the authors over-expressed SIGIRR in Jurkat

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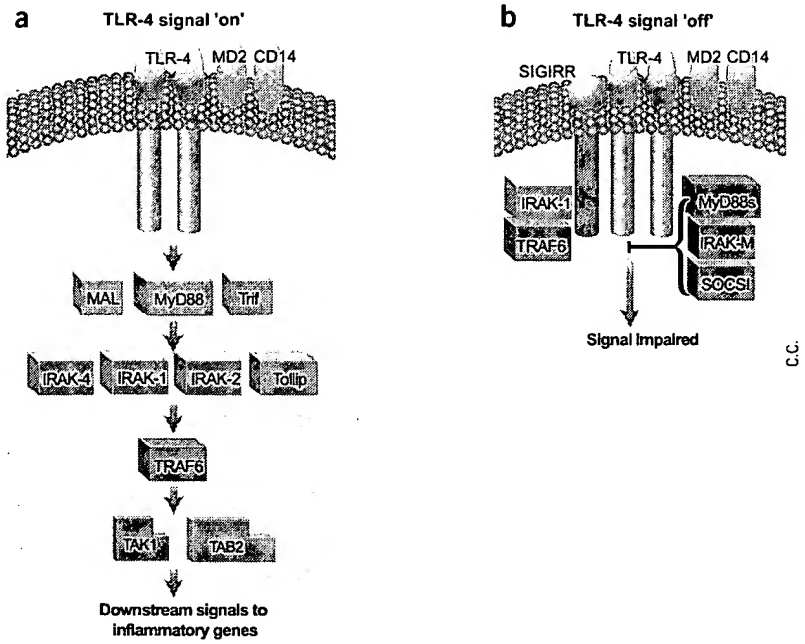

Figure 1 The 'on' and 'off' of TLR4 signal transduction. (a) TLR4 signal transduction is initiated by the recruitment of three TIR domain-containing adapters: MyD88, Mal and TRIF. IRAK-4 is then recruited to the complex, where it phosphorylates IRAK-1, leading to TRAF-6 engagement and the activation of the kinase TAK-1, in a complex with TAB2. IRAK-2 and Tollip are also involved in TLR4 signal transduction, although their precise involvement is not fully worked out. The transcription factor NF-κB and several MAP kinase cascades lie downstream and lead to enhanced transcription and translation of inflammatory and immune genes. (b) Negative regulators of TLR4 signal transduction. SIGIRR is a transmembrane protein with a TIR domain, which is recruited to TLR4 and blocks signaling by sequestering IRAK and TRAF-6. MyD88s is a splice variant of MyD88, which prevents IRAK-4 recruitment. IRAK-M inhibits TLR4 signaling by preventing dissociation of IRAK from the signaling complex. It is likely that a balance between activation and inhibition of signaling is responsible for the output from TLR4 and other members of the TLR–IL-1R family such as IL-1RI, which show similar negative controls.

and HepG2 cells and demonstrated that it inhibited NF-κB activation by IL-1 and IL-18. Clear indications for an inhibitory function came when they constructed SIGIRR-deficient mice, which were shown to be hyper-responsive to LPS and IL-1 but not TNF, whose receptor is not in the TLR–IL-1R superfamily. Primary kidney epithelial cells and splenocytes prepared from these mice were also hyper-responsive to IL-1 and LPS in terms of NF-κB and JNK activation. SIGIRR-deficient splenocytes were shown to be hyper-responsive to CpG DNA, in terms of proliferation, indicating the inhibitory effects of SIGIRR may be broader than its effects on IL-1R and TLR4. No difference was found between SIGIRR-deficient and wild-type macrophages, consistent with the lack of expression of SIGIRR in these cells. Finally, the authors show that SIGIRR is associated with IL-1RI, TLR4, TLR5, TLR9 and TRAF-6. IL-1 treatment promoted the association of SIGIRR with IL-1RI and also led to the formation of a complex between SIGIRR, IL-1R-associated kinase (IRAK) and TRAF-6. These mechanistic studies indicate that SIGIRR works by being recruited through its TIR domain to the TIR domain of target receptors, where it might sequester the key signalling proteins IRAK and TRAF-6, and prevent signal propagation (Fig. 1). The evidence for this mechanism is, however, circumstantial and it is possible that SIGIRR launches a negative signal.

SIGIRR seems to act as a pan-inhibitor of the receptor superfamily, although further evidence in support of this notion is needed, in particular with regard to TLR9 and TLR5. The other known endogenous inhibitors of TLR signalling are MyD88s, IRAK-M and SOCS-1 (refs. 9–11). MyD88s, which is a splice variant of MyD88, prevents IRAK-4 recruitment[9]. IRAK-M also interferes with IRAK function by preventing dissociation of IRAK from the signalling complex[10]. The mechanism of action of SOCS1 is not known. Mice deficient in IRAK-M or SOCS1 have a very similar phenotype to that of SIGIRR-deficient mice in terms of LPS hyper-responsiveness, and both IRAK-M and SOCS-1 are important in LPS tolerance, in which cells become unresponsive to LPS. SIGIRR may therefore also be involved in this process, particularly in epithelial cells. SIGIRR may in fact be important for tolerance to microbes in the gut and other epithelial tissues.

The findings of Wald *et al.* are of interest for the development of inhibitors of TLR function, which could be used to treat sepsis and inflammatory diseases. The proposed interaction between the TIR domain of SIGIRR and the TIR domains of target receptors indicates that it might be possible to interfere with the TIR domain function with inhibitors. An inhibitor based on the so-called BB loop in the TIR domain has been developed[12]; it seems to act by preventing recruitment of adapters to receptor TIR domains. Perhaps SIGIRR has a similar obscuring effect. Future studies will need to determine the mechanism of SIGIRR recruitment and to define what it is about its TIR domain that makes it inhibitory. A final issue regarding mechanism concerns a ligand for SIGIRR. Even though it only has one Ig domain, SIGIRR may still have a ligand, which could be one of the IL-1 paralogs. No ligand was used in the study by Wald *et al.*, however, although over-expression of SIGIRR might have effects similar to ligand engagement. Like other receptor systems in immunity, the TLR–IL-1R superfamily now has an inhibitory member. Further analysis will continue to improve our understanding of the functions of this fascinating and essential set of receptors in innate immunity and inflammation.

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Extracellular and intracellular decoys in the tuning of inflammatory cytokines and Toll-like receptors: the new entry TIR8/SIGIRR.

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Following the identification of the interleukin (IL)-1 type II receptor as a prototypic decoy receptor, nonsignaling receptors with decoy functions have been identified for members of the IL-1/IL-18, tumor necrosis factor, IL-10, and IL-13 receptor families. Moreover, the silent receptor D6 is a promiscuous decoy and scavenger receptor of inflammatory chemokines. Th type II IL-1 decoy receptor also acts as a dominant-negative molecule. Intracellular pathways of inhibition of IL-1 and Toll-like receptor (TLR) signaling have been identified. In particular, recent results suggest that the Toll/IL-1 receptor (TIR) family member TIR8, also known as single immunoglobulin IL-1-related receptor (SIGIRR), is a negative regulator of IL-1 and TLR signaling. Thus, extracellular and intracellular decoys tune the activation of members of the IL-1/TLR receptor family.

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Unique pattern of expression and inhibition of IL-1 signaling by the IL-1 receptor family member TIR8/SIGIRR.

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TIR8, also known as single Ig IL-1R-related molecule (SIGIRR), is a membe of the IL-1 receptor family. The present study was designed to investigate th expression and function of TIR8. TIR8 was mainly expressed in mouse and human epithelial tissues such as kidney, lung and gut. Resting and activated and B lymphocytes and monocytes-macrophages expressed little or no TIR8, with the exception of the mouse GG2EE macrophage line. In the kidney, the organ with highest mRNA levels, TIR8 expression was confined to epithelial cells and, in situ, to tubular epithelium. A variety of signals failed to regulate TIR8 expression, but LPS reduced TIR8 mRNA transcripts. An NF-kB drive reporter system was used to investigate the function of TIR8. TIR8 did not activate NF-kB expression alone or in concert with IL-1R1. In contrast, TIR inhibited signaling from the IL-1R complex. Inhibition required the intracellular portion of TIR8 but the extracellular domain was dispensable fo blocking activity. Thus, TIR8 is a unique member of the IL-1R family, with distinct pattern of epithelial expression, including the kidney and mucosae, and an inhibitory function on IL-1 signaling. Copyright John Libbey Eurotex 2003.

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